PHYTOPATHOLOGY

VOLUME XV NUMBER 7

JULY, 1925

THE TRANSMISSIBLE LYTIC PRINCIPLE (BACTERIOPHAGE) IN RELATION TO PLANT PATHOGENES

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With Plates IX to XII

The work of d'Herelle (1) with various animal pathogenes, especially *Bacillus dysenteriae* Shiga, has established the existence of a transmissible lytic principle[1] effective against certain bacterial strains. d'Herelle has named the principle "the bacteriophage," and he believes his experiments show the existence of an ultra-microscopic parasite, *Bacteriophagum intestinale* d'Herelle, (3) living as an obligate parasite on bacteria. The phenomenon of lysis of bacterial cultures into which the active filtrate has been introduced is explained as due to a single species of organism capable of growth and capable of attacking a wide range of bacterial species. Material investigated by him has always been derived from the intestinal tracts of animals, and the lines of investigation have proceeded upon the assumption of an animal source of the lytic principle. The organisms used have been animal pathogenes.

The work of d'Herelle has stimulated a large amount of study of this phenomenon, and many organisms pathogenic to man and lower animals have been studied in this regard. Very little has as yet been published on the relation of the transmissible lytic principle to the bacterial pathogenes of plants.

Gerretsen and Sack, and Söhngen and Gryns (4), both groups working independently at first and later in cooperation, probably were the first to record the d'Herelle phenomenon in relation to bacteria associated with

[1] In this article the terms commonly used in the literature with reference to the d'Herelle phenomenon have been employed. The writers feel that their experiments have not been of the type to bear directly on the moot point of whether lysis is brought about by a living ultra-microscopic organism or whether some other explanation is more tenable. The term "transmissible lytic principle" has been employed following common usage in this country (2).

plants. These investigators were successful in isolating lytic principles from nodules of various leguminous plants. These were specific for the bacteria of the legume concerned. Isolations of the lytic principles were also made from roots and stems, but not from leaves of the legumes; from garden and field soil, but not from heath or forest soil. The lytic principles were found to withstand desiccation, as well as heating at from 60° to 65° C. for fifteen minutes; and they were at least eight times as resistant to ultra-violet light as were the bacteria upon which they were effective.

Independently, Mallmann and Hemstreet (5) also demonstrated the d'Herelle phenomenon, using an organism obtained from a plant source. These workers obtained what they call an "inhibitory substance" from cabbage which had been rotted by fluorescent organisms. They were able to demonstrate marked inhibition of growth with extremely high dilutions of the filtrate, but were not able to demonstrate actual lysis. They present evidence of the increase of activity upon successive transplantings and filtrations and they obtained inhibition with a dilution of 1 : 100,000,000,000. The bacterial organism isolated by Mallmann and Hemstreet produced a slow rotting of cabbage, several weeks being required for complete liquefaction. It is stated by them that the organism belongs to the fluorescent group rather than to *Bacillus carotovorus* and its allies. These workers further tested the "inhibitory substance" against known plant pathogenes, *Bacillus carotovorus*, Spieckerman's bacillus, and the "potato rot bacillus"; and with the first two, except where large amounts of filtrate were used, no inhibition was noted. The "potato rot bacillus" was not inhibited at all.

The work of Mallmann and Hemstreet very evidently opens up a very interesting field of experimentation from the point of view of plant pathology. In order to ascertain whether the d'Herelle phenomenon could be demonstrated with known plant pathogenes, such as *Bacillus carotovorus* Jones, *B. atrosepticus* vanHall, and *Bacterium tumefaciens* EFS. and Towns, and to determine the rôle played in pathogenesis, experiments were undertaken early in 1924. Following the methods of d'Herelle[2] an apparently sterile filtrate was obtained from a carrot which had decayed with typical soft rot when stored in sand in a basement. When various quantities of this filtrate (1 drop, 10 drops, 2 cc.) were placed in tubes contain-

[2] A portion of a rotted carrot, and adhering sand, was incubated in 25 cc. of standard beef broth for 24 hours. The mixture was filtered through filter paper and then through filter paper lined with infusorial earth. The clear filtrate was then refiltered through a Pasteur bougie (L7) and an apparently sterile filtrate was obtained which was demonstrated to contain the transmissible lytic filtrate. Subsequent filtrates were obtained from pooled cultures of an earlier series of tests. Alkaline Liebig beef broth (pH 8) was used in all tests unless other medium is mentioned.

ing suspensions of *Bacillus carotovorus* in alkaline Liebig beef extract broth, an inhibiting effect on the growth of the organism was noted after three days' incubation at 25° C. All tubes containing the filtrate were less turbid than the check.

Similar results were obtained from filtrates obtained from river water and from soil which had been drenched with heavy suspensions of pure cultures of *B. atrosepticus*. This soil had been used in experiments to determine whether infested soil is a source of the black leg disease of potatoes (7).

INCREASE IN POTENCY OF SUCCESSIVE FILTRATES

The potency of the lytic principle was found to be increased after repeated association with susceptible organisms. The original material showed only inhibitory effects, whereas, after exposure to susceptible organisms and refiltering four times, the activity of the filtrate so obtained was so increased that it was effective in causing inhibition up to a dilution of 1:100,000,000 and capable of causing distinct clearing of suspensions in the lower dilutions. In the case of the filtrate of four passages the effect was apparent after fourteen hours, whereas the first filtrate did not show inhibition effects until after three days.

After the same material had undergone exposure to susceptible organisms and refiltering four times more, the two filtrates were compared by means of a dilution experiment. Two series of tubes, each containing 9 cc. of alkaline beef broth, were treated as follows: One cc. of the material of the fourth passage was added to the first tube of one series and was mixed thoroughly by shaking. A dilution of 1:10 was thus obtained. By means of another sterile pipette, 1 cc. of this dilution was added to the next tube of the series, making a dilution of 1:100. The process was repeated, using a fresh sterile pipette each time until a dilution of 1:100,000,000 was obtained. The second series was treated similarly, using at the start 1 cc. of the material of the eighth passage. Each tube was then inoculated with 1 cc. of a suspension of *B. carotovorus* made by diluting 5 cc. of a 3-day-old broth culture with 25 cc. of sterile broth. Two tubes of sterile broth were inoculated with the same quantity of organisms to serve as checks. The invigorating effects of the additional exposures to the susceptible organism can be seen from table 1.

It is seen from the above results that the effect of the material of the eighth passage is more pronounced than that of the fourth passage. The inhibition effects upon growth were evident earlier and lasted longer in the case of the eighth filtrate. The increase of potency of the eighth filtrate can be seen by comparing figures 1 and 2 of plate IX. The relative clear-

TABLE 1.—*Degree of lysis with Bacillus carotovorus: Comparison of the lytic principle of the 4th and 8th passage*

Times invig-orated	Incuba-tion period	1 : 10	1 : 100	1 : 1000	Dilutions		1 : 1 million	1 : 10 million	1 : 100 million	1 : 1000 million	check
					1 : 10,000	1 : 100,000					
4	1 day	+	+	++	+++	+++	+++	++	–	–	–
8	1 day	++++	++++	++++	++++	++++	++++	+++	+	–	–
4	2 day	+++	++	+++	+++	++	+	++	–	–	–
8	2 day	+++	+++	++++	++++	++++	++++	++	+++	–	–
4	3 day	++++	+++	+++	+++	++++	++++	++	–	–	–
8	3 day	++	+++	+++	+++	+++	++++	++	++++	–	–
4	4 day								+	+	–
8	4 day								+++	–	–
4	8 day								+	+	–
8	8 day								+++	+	–
4	14 day	+	+	+	+	+	+	+	+	+	–
8	14 day	++++	++++	++++	++++	++++	+++	++	+	+	–

Note: Readings on 4th and 8th days were made only for the highest dilutions.

– No inhibitory or lytic effect, medium very turbid.

+ Slight inhibition or lysis effect, medium turbid.

++ Medium inhibition or lysis, medium slightly turbid.

+++ Strong inhibition or lysis, medium very slightly turbid.

++++ Very strong inhibition or lysis, medium very clear.

ness of medium as shown in the photographs indicates that the inhibition to growth is not so strong in the tubes labelled "F 13" (four passages) as in the series of "F 23" (eight passages). The photographs were taken after an incubation period of three days.

MICROSCOPIC STUDY OF CULTURES

Examination of a great many hanging drop cultures made from normal cultures and those containing the lytic principle showed certain characteristic developments in the "phage" cultures. Decrease in motility, agglutination, and malformation of the organisms were constantly found in material from culture tubes to which the lytic principle had been added. The normal cultures did not show these peculiarities. These observations were made upon the three plant pathogenes already named. The shapes of malformed organisms from "phage" cultures varied considerably. Some individuals were much elongated, others were elongated and swollen, some were bulged at one end, while still others were short and markedly distended at the middle to a spindle shape. Enlarged spherical forms were observed also, but none of these were observed to burst as is reported by d'Herelle.

Some interesting observations were made in sets of hanging drop cultures which were made from a certain dilution experiment. In this experiment dilutions of 1:2,000, 1:20,000, 1:200,000, and 1:2,000,000 were made. The first set of hanging drop cultures was made from these dilutions immediately after the experiment was started, while the second set was made from the same tubes after four days incubation. Examination of the hanging drops of the first set after a period of twenty-four hours showed agglutination and malformation in the slides made from the 1:200, 1:2,000 and 1:20,000 dilutions. In the highest dilutions, 1:200,000 and 1:2,000,000, the organisms were actively motile and appeared no different from the check. The hanging drops of the second set were examined immediately after they were made and, in the case of the lower dilutions, were like the hanging drops of the lower dilutions of the first set. In the hanging drops of the two highest dilutions, however, agglutination was observed. The same set of slides (4-day) were again examined after twenty-four hours, and it was found that the organisms had become malformed, while the corresponding hanging drop cultures of the first set remained like the checks, apparently normal. In the meantime the test-tube cultures of the two highest dilutions had become much less turbid than the check, indicating the presence of the lytic principle even after high diluting. These observations seem to indicate that the lytic principle is corpuscular and increases in the course of a period of culture. It is plausible to ex-

plain these results by assuming that in the 1 : 200,000 and 1 : 2,000,000 dilutions few particles of the lytic principle were present at the beginning of the incubation period, and when hanging drops were made from these by means of a straight needle none of the particles happened to be carried over, hence the bacteria in these drops remained normal. However, after four days' incubation the number of the particles had increased to such an extent that they had got the upper hand, so to speak, of the bacteria, as evidenced by the agglutination observed, and on further incubation caused malformation and behavior typical of the d'Herelle phenomenon. The development of agglutination seems to presage the subsequent inhibition effects in the cultures.

TEMPERATURE RELATIONS

Work with the animal pathogenes carried on by other workers had been done usually at 37° C., which is near the optimum for the organisms used. The optimum of the organisms used in our tests lies much lower. Preliminary experiments had shown a marked difference in the activity of the lytic principle between culture tubes incubated at 37° C. and room temperature. These led to further tests on the effects of various temperatures on the activity of the lytic principle. For this purpose an experiment was conducted using a nine-chamber differential thermostat in which the average temperature range was from 7.8° C. to 36.1° C. with a variation of not more than 1½ degrees. One broth culture containing the lytic principle and one check culture of *B. carotovorus* were placed in each chamber and incubated for six days with the following results (Table 2).

Very little difference was observed between the check and the "phage" tubes at the two highest and the two lowest temperatures. The checks showed a very poor growth at these temperatures, while at temperatures more nearly optimum for the organism the check tubes became turbid in two days while the "phage" tubes remained clear. Evidently conditions which are favorable for the growth of the organism likewise are essential for the development of strongest lysis.

SPECIFICITY OF THE LYTIC PRINCIPLE

d'Herelle has suggested that the bacteriophage is polyvalent and has postulated one organism which is capable of attacking all species of bacteria. Since the early work, strains of bacteriophage, in the terminology of d'Herelle, both polyvalent and univalent have been isolated by various workers. The isolation from carrot made by us was tested out against several plant and animal pathogenes.

TABLE 2.—*Effect of various temperatures on lytic action: Tests with Bacillus carotovorus; incubation period, six days*

No. of chamber	Average temp. C.	Results	
		Plus filtrate	Check
1	7.8	no growth	no growth
2	11.7	little growth	little growth
3	16.8	++++	–
4	19.5	++++	–
5	22.0	++++	–
6	23.4	++++	–
7	24.7	+++	–
8	26.7	++	little growth
9	36.1	(?)	no growth

– No lytic effect, medium very turbid.
+ Slight lytic effect, medium turbid.
++ Medium lytic effect, slightly turbid.
+++ Strong lytic effect, very slightly turbid.
++++ Very strong lytic effect, very clear.

Numerous tests of the lytic principles isolated by the writers against *Bacterium tumefaciens*, *Bacillus carotovorus*, and *B. atrosepticus* have shown that these are effective against all, but not equally. The lytic principle has seemed to be most effective against *Bact. tumefaciens* (from peach, obtained from Dr. Smith), followed in order by *Bacillus carotovorus* (Kotila isolation) and *B. atrosepticus* (Kotila isolation). Table 3 and plate X show the effect of the lytic principle isolated from carrot in various dilutions against these organisms. Although differences in effectiveness of the lytic principle were observed, no such marked specificity was apparent as noted by Gerretsen et al. (3).

A series of dilutions ranging from 1:10 to 1:100,000,000, using potato broth to which 1 per cent dextrose was added and whose reaction was neutral, showed lysis of *Bacillus carotovorus* in all except the greatest dilution. These results are at variance with reports in the literature, which have indicated that the presence of sugar prevented lysis.

In other tests standard beef broth has been used without the addition of the excess of sodium hydroxide. Comparative dilution tests have shown no marked difference in lysis between the ordinary standard beef broth and the strongly alkaline broth. This result also is at variance with some reports in the literature.

Apparently sterile broth containing the lytic principle (9th passage) was sealed in a flask with a paraffined stopper. After 5½ months it showed no evidence of contamination and it was again tested. The filtrate

TABLE 3.—*Dilution test with lytic principle: Fourth passage filtrate tested against 3 organisms; room temperature*

Organism	Interval	Dilutions								Check No lytic principle
		1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	1:10 million	1:100 million	
Bacillus carotovorus (54)	17 hrs.	+++	+++	+++	+++	–	–	–	–	–
	41 hrs.	++++	++++	+++	+++	+++	+++	++++	+++	–
	3 da.	++++	++++	++++	++++	++++	++++	++++	+++	–
	5 da.	–*	–*	–*	–*	–*	–*	–*	–*	–
	10 da.	–	–	–	–	–	–	–	–	–
Bacillus atrosepticus (10)	17 hrs.	+++	+++	+++	–	–	–	–	–	–
	41 hrs.	++++	++++	++++	+++	+++	+++	++	+	–
	3 da.	+++	++++	++++	+++	+++	+++	+++	+++	–
	5 da.	+++	+++	++++	++++	++++	++++	+++	+++	–
	10 da.	–	–	–	–	+++	+++	++++	–	–
Bact. tumefaciens (146)	17 hrs.	++++	+++	+++	+++	–	–	–	–	–
	41 hrs.	++++	++++	++++	++++	++++	++++	+++	+++	–
	3 da.	++++	++++	++++	++++	++++	++++	+++	+++	–
	5 da.	–	++++	++++	++++	++++	++++	+++	+	–
	10 da.	–	++++	++++	++++	++++	++++	++++	++++	–

* Cloudy, but distinctly less than check; lower dilutions, showing slightly more than higher dilutions.

– No inhibitory or lytic effect, medium very turbid.

+ Slight inhibition or lysis effect, medium turbid.

++ Medium inhibition or lysis, medium slightly turbid.

+++ Strong inhibition or lysis, medium very slightly turbid.

++++ Very strong inhibition or lysis, medium very clear.

was not so active as when put aside but was capable of inhibiting growth. It was not capable of causing clearing except with *Bacillus carotovorus*.

In this test various other bacterial organisms were included, many representing organisms not previously used, and some representing other strains of those previously used in the tests. For comparison with the results of other workers, three animal pathogenes were included. The results of this test are given in table 4.

TABLE 4—*Test of lytic principle against various organisms: Filtrate (9th passage) used after 5½ mo. storage, 1 drop per tube*

Organism	Plus lytic principle		Check	
	2–11–25	2–13–25	2–11–25	2–13–25
Bacillus carotovorus (Bot. Exp. Strain)	+	+	—	—
B. carotovorus (Strain 54)	+++	++	—	—
B. atrosepticus "30"	—	+	—	—
Bact. pruni	—	—	—	—
Bacillus melonis	—	—	—	—
Bact. tumefaciens (from Hop; courtesy Dr. Smith)	—	—	—	—
*Bacillus amylovorus**	—	—	—	—
B. typhosus	—	±	—	—
B. coli	—	±	—	—
B. dysenteriae (Shiga)	—	—	—	—

* *B. amylovorus* had shown slightly inhibited growth in test 4–8–24.

— No inhibitory or lytic effect, medium very turbid.

+ Slight inhibition or lysis, medium turbid.

++ Medium inhibition or lysis, medium slightly turbid.

+++ Strong inhibition or lysis, medium very slightly turbid.

++++ Very strong inhibition or lysis, medium very clear.

Several additional passages and filterings of the material have been made with a rather prompt increase of the potency of the lytic principle. In these tests it has been noteworthy that *Bacterium tumefaciens*, which in the first tests was completely and permanently lysed, has in the later work been only slightly affected. Tubes of this organism to which the lytic principle is added show inhibition of growth for two or three days and then definite pellicle formation occurs, in marked contradistinction to the earlier results. It should be noted that the strain of *Bact. tumefaciens* used is not the same one used in the first experiments.

The additional passages have to date not increased the activity toward any of the animal pathogenes, *Bacillus typhosus* showing consistently only slight inhibition in growth in those tubes to which the lytic principle has been added.

Our experience indicates that while the lytic principle may show activity against a considerable group of organisms, it varies in its effectiveness and seems more active against certain strains than against others. The failure of the lytic principle to lyse the Shiga organism is interesting. The lytic principle seems to lose its potency upon standing.

To test out further the lytic principle obtained from a plant source, cultures of *Bacterium pullorum* Rettger and Harvey and *Bacterium sanguinarium* Moore were obtained through the courtesy of Dr. H. J. Stafseth of the Department of Bacteriology, Michigan Agricultural College. This work was done with filtrates that were very active against *Bacillus carotovorus*. No difference in turbidity of the checks and the cultures to which the lytic principle was added was observed in any of the numerous trials which were made. Invigoration of the material after the methods recommended by d'Herelle (1) were attempted. The two animal pathogenes in some cases were grown in the presence of the lytic principle and in others in the presence of the lytic principle together with the various plant pathogenes against which it was effective. The filtrates from these cultures when tested out against the two animal pathogenes yielded negative results. Similar negative results with these organisms were obtained by Mallmann[3] with his isolations.

RELATIONS TO PLANT PATHOLOGY

The first important rôle which has been suggested by our experiments is concerned with the decline of certain bacterial organisms in the soil. Various workers have noted the decline if not the disappearance of various bacterial organisms in soil, as for example, *Pseudomonas citri* (6). Of special interest is the apparent disappearance of the organism causing potato blackleg (*Bacillus atrosepticus*) from soil in a comparatively short time. The writers have reviewed in another publication the work of Morse, Rosenbaum and Ramsey, and Murphy, as well as given the results of their tests (7). It has been possible to isolate from loam soil which had been drenched heavily with cultures of *Bacillus atrosepticus* a lytic principle which has behaved essentially as the lytic principle isolated from rotted carrot. Similarly a lytic principle has been isolated from river water, which was also effective against *B. atrosepticus* and other plant pathogenes. The writers would conclude that *Bacillus atrosepticus* along with other plant pathogenes, *B. carotovorus* and *B. tumefaciens*, is not refractory toward lysis, that the lytic principles are widely distributed in nature, and that lysis may play an important rôle in decimating or rendering non-virulent these particular organisms. Furthermore, the increase

[3] Verbal communication.

of potency of the lytic principle when exposed to susceptible organisms seems to indicate that the range of organisms concerned in this phenomenon is large. It must be borne in mind that variations in activity with various strains of organisms seem to exist.

The lytic principles can play an exceedingly important rôle in preventing normal infection. Since the early experiments of Jones with the soft rot organism, the rapid and complete rotting of moist carrot disks by *Bacillus carotovorus* has become a common classroom demonstration. Similarly, the rotting of potato slices after inoculation with *Bacillus atrosepticus* follows promptly and with production of a characteristic decay if the tuber slices are kept moist. It is interesting to compare the normal results with those obtained when the lytic principle is introduced as a factor in the experiments.

Fresh, crisp carrots were carefully washed and disinfected with corrosive sublimate. These were sliced into disks with a sterile knife and the disks placed on filter paper in small individual moist chambers freshly steamed, which were in turn held in a larger moist chamber. Five sets of four carrot slices each were used and treated as follows: Series 1. Each slice was inoculated with a loop-drop of sterile nutrient beef broth. Series 2. Each slice was inoculated with a loop-drop of a 48-hour broth culture of *B. carotovorus*. Series 3. Each slice was inoculated with a loop-drop of a mixture made by adding 1 cc. of the 48-hour broth culture used in Series 2 with an equal quantity of the lytic principle obtained originally from carrot. Series 4. This was the same as Series 3, except that the inoculation was made after six hours incubation of the organism with the lytic principle. Series 5. A loop-drop of the lytic principle was spread over the surface of each carrot slice after which they were inoculated with a loop-drop of the 48-hour culture of *B. carotovorus*. In every case the slices were placed in the moist chambers immediately after inoculation. After three days' incubation at room temperature the following results obtained (Table 5 and Plate XI).

TABLE 5—*Protective effect of the lytic principle against Bacillus carotovorus infection: Tests with carrot slices; 3-day period*

Series	Treatment	Results
1	Check, sterile broth	– – – –
2	*B. carotovorus*	+ + + +
3	*B. carotovorus* plus lytic principle, inoculated immediately	– – – –
4	*B. carotovorus* inoculated after 6 hours	– – – –
5	Lytic principle spread on slice just before inoculation	– – – –

– No rotting. + Rotting.

The results from this test were precise and definite. *Bacillus carotovorus* promptly and completely rotted the carrot slices in the check series, whereas none of the slices which had the lytic principle brought in contact with the organism rotted at all. Even with the rather uneven distribution of the lytic material such as would result from spreading a loop-drop over the slice with a platinum needle, rotting was prevented.

In experiments using potato slices and *Bacillus atrosepticus* as the pathogene, similar protective action by the lytic principle was demonstrated. Sound potato tubers were washed and disinfected with corrosive sublimate. These were sliced and placed in individual moist chambers lined with filter paper. A loop-drop of the filtrate containing the lytic principle was spread over the cut surface of each of five slices, two slices being used as checks. Each slice was then inoculated with a loop-drop of *B. atrosepticus* (24-hour culture). The results of this test are shown in table 6a and plate XII.

TABLE 6—*Protective action of lytic principle against B. atrosepticus: Test with potato slices in moist chamber; 3-day period*

a

	Treatment	Result	
Slice 1	ck.	+	Characteristic rot
2	ck.	+	Characteristic rot
3	Plus 1 loop lytic filtrate	–	No rotting
4	" 1 loop lytic filtrate	+	Characteristic rot
5	" 1 loop lytic filtrate	–	No rotting
6	" 1 loop lytic filtrate	–	No rotting
7	" 1 loop lytic filtrate	–	No rotting
On 3rd. day, Nos. 3, 5, and 7 were reinoculated			No rotting developed

b

Tuber 1,			
Slice 1	ck.	+	Characteristic rot, 15 mm. diam.
2	Plus 1 loop lytic filtrate	+	Characteristic rot; one spot 5 mm. diameter, one 1 × 3 mm.
3	" 1 loop lytic filtrate	–	No rot
4	" 1 loop lytic filtrate	–	No rot
Tuber 2,			
Slice 5	" 1 loop lytic filtrate	–	No rot
6	" 1 loop lytic filtrate	–	No rot
7	" 1 loop lytic filtrate	–	No rot
8	ck.	+	Characteristic rot; one area 10 × 20 mm., another 7 × 15 mm.

– No rotting. + Rotting.

The experiment was repeated following the same plan with the results that are shown in table 6b.

The lytic principle when placed upon potato slices operates to prevent the normal process of infection with *B. atrosepticus*. The rotting of a single tuber slice stroked with a loop of lytic filtrate in each of the two tests is not believed to be significant, as by the method employed even distribution of the lytic material can hardly be obtained. When the loop of material is placed upon the slice, the bulk of the drop is deposited at once and spreading must be accomplished by stroking. This militates against reaching all parts of the surface. On the other hand, when the bacteria are placed upon the tuber, the heavy deposit of the bacteria at some one place greatly favors infection at that point.

The writers wish to record their preliminary experiments in using the lytic principle as a protective agency in preventing infections. We believe this work opens up some possibilities in the matter of plant disease control. We believe the relations of the lytic principle to life processes of plant pathogenes needs full and complete investigation. Furthermore, the lytic principle may be a factor playing an important rôle in the infection phenomena in plant diseases of bacterial nature.

SUMMARY

1. The d'Herelle phenomenon has been demonstrated with various plant pathogenes. A transmissible lytic principle (bacteriophage) has been obtained from rotted carrot, from soil, and from river water, which was found to be effective in very high dilutions in causing inhibition of growth of various bacteria and definite lysis in stronger concentrations. The organisms chiefly used were *Bacillus carotovorus*, *B. atrosepticus*, and *Bacterium tumefaciens*.

2. The potency of these filtrates was increased after a number of passages with a susceptible organism. The lytic principle of eight passages was more effective than the same material of the 4th passage, producing lysis even in dilutions as high as 1 : 100,000,000.

3. The lytic effects are not static but vary slightly from day to day, especially in tubes with less active lytic principle and with higher dilutions.

4. Loss of motility, malformation, and agglutination are characteristic occurrences in cultures to which the lytic principle has been added.

5. Some evidence that the lytic principle is made up of corpuscles which increase in number upon incubation with a susceptible organism is given. Agglutination seems to be an early evidence of the workings of the lytic principle.

6. Temperature tests, using a range of temperatures from 7.8° C. to 36.1° C., showed that the activity of the lytic principle is greatest at those

temperatures most favorable for the growth of the susceptible organism. The experiments with the plant pathogenes were carried out chiefly at room temperature (25° C.).

7. The lytic principle was polyvalent toward *Bact. tumefaciens*, *Bacillus carotovorus*, and *B. atrosepticus*, and but slightly active against certain other organisms, such as *B. amylovorus* and *B. typhosus*. It was not active against *Bacillus dysenteriae* Shiga, *Bacterium pullorum*, and *Bact. sanguinarium*. Its activity varied with the strains of the organism tested.

8. The potency of the filtrate was lessened after 5½ months storage in a sealed flask.

9. The occurrence of lytic principles in nature is believed to be widespread, and it is suggested that they play an important rôle in the decline of certain bacterial organisms in the soil.

10. When the lytic principle was spread upon slices from susceptible plants (carrot, potato), infection by *Bacillus carotovorus* and *B. atrosepticus*, respectively, was in whole or in part prevented. This protective action may be of importance in infection phenomena as well as of importance in plant disease control.

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LITERATURE CITED

1. D'HERELLE, F. The bacteriophage, its role in immunity. Tr. by G. H. Smith. 279 p. Baltimore, 1922.
2. HADLEY, P. B. Transmissible lysis of *Bacillus pyocyaneus*. Jour. Infect. Dis. **34**: 260. 1924.
3. D'HERELLE, F. Technique de la recherche du microbe filtrant bactériophage. Compt. Rend. Soc. Biol. Paris **18**: 1160–1162. 1918.
4. GERRETSEN, F. C., A. GRYNS, J. SACK, and N. L. SÖHNGEN. Das Vorkommen eines Bacteriophagen in dem Wurzelknöllchen der Leguminosen. Cent. Bakt. II. **60**: 311–316. 1923.
5. MALLMANN, W. L. and CARL HEMSTREET. Isolation of an inhibitory substance from plants. Jour. Agr. Res. **28**: 599–602. 1924.
6. FULTON, H. R. Decline of *Pseudomonas citri* in the soil. Jour. Agr. Res. **19**: 207–223. 1920.
7. KOTILA, J. E. and G. H. COONS. Investigations on the blackleg disease of potato. Mich. Sta. Tech. Bul. 67. 1925.

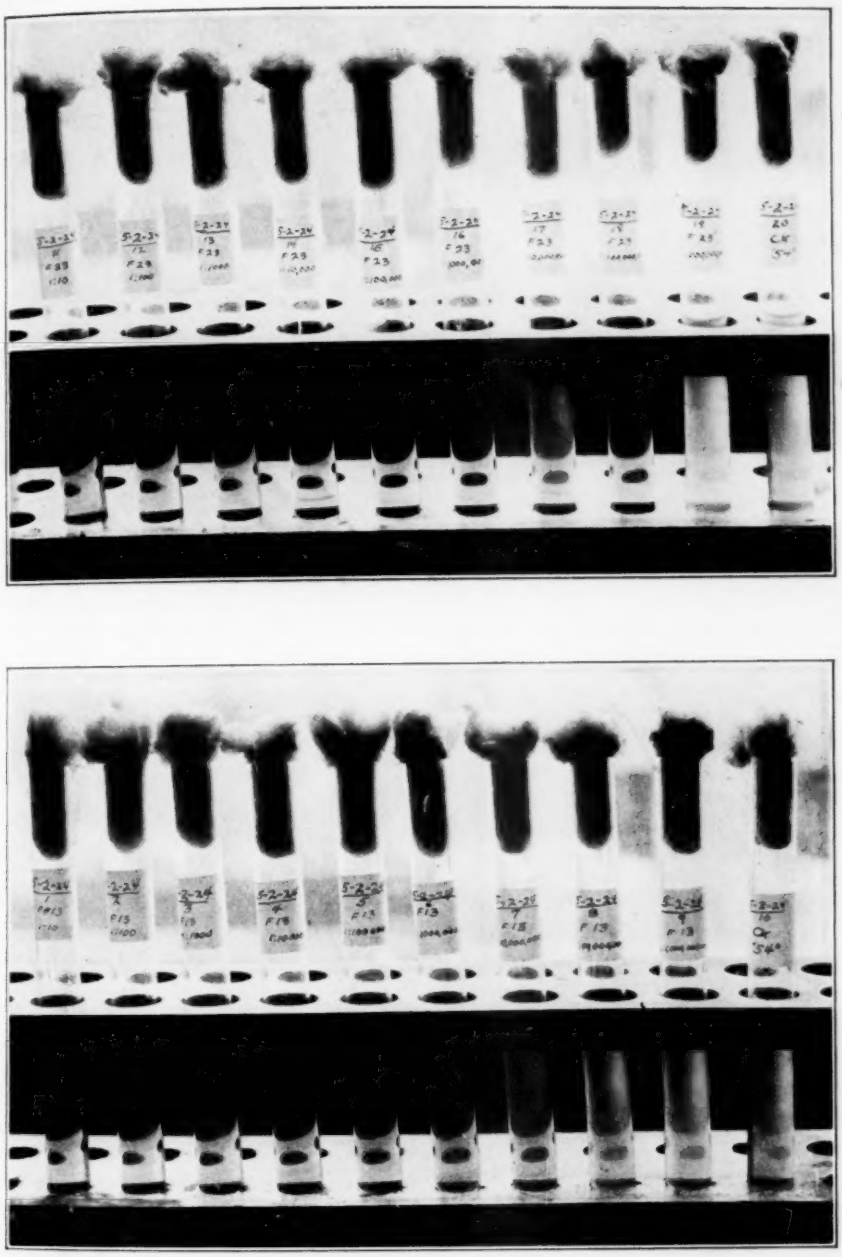

PLATE IX, Figs. 1 and 2. Effect of lytic principle on *Bacillus carotovorus*. (Fourth and eighth serial filtrates compared.)

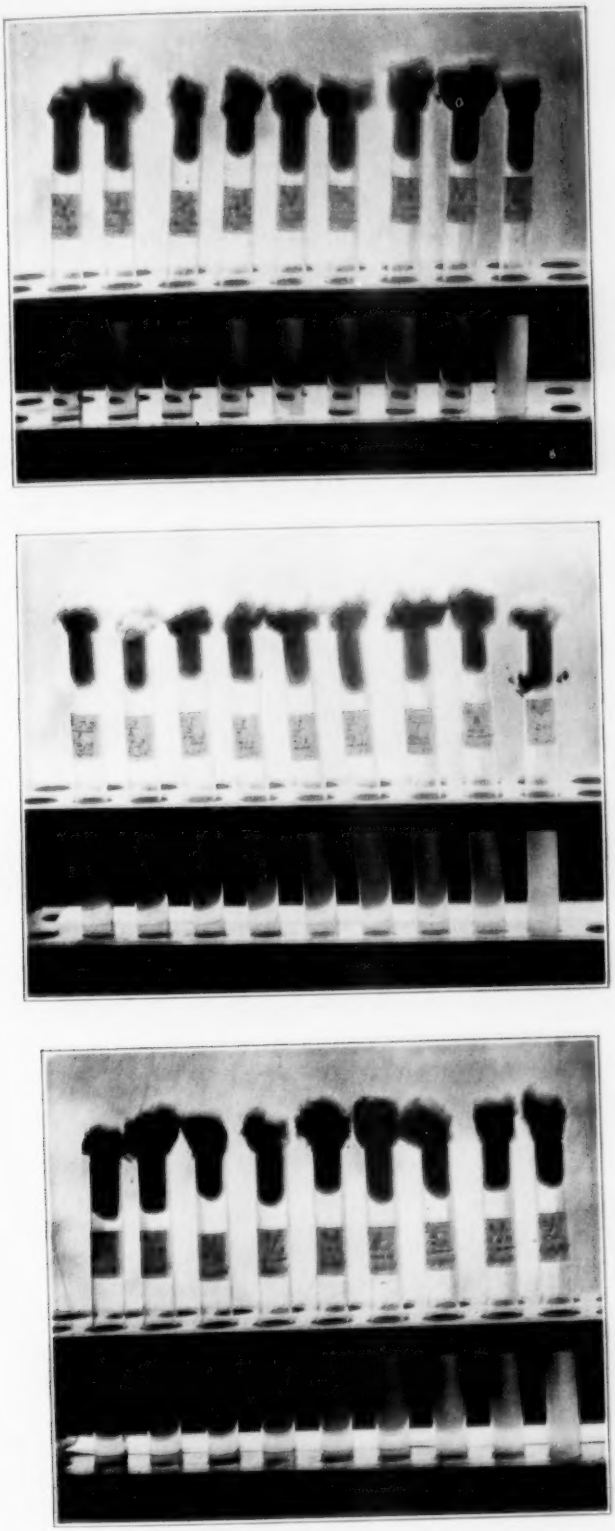

PLATE X, Figs. 1, 2 and 3. Comparison of the effect of lytic principle on *Bact. tumefaciens*, *Bacillus carotovorus*, and *B. atrosepticus*; 3-day period.

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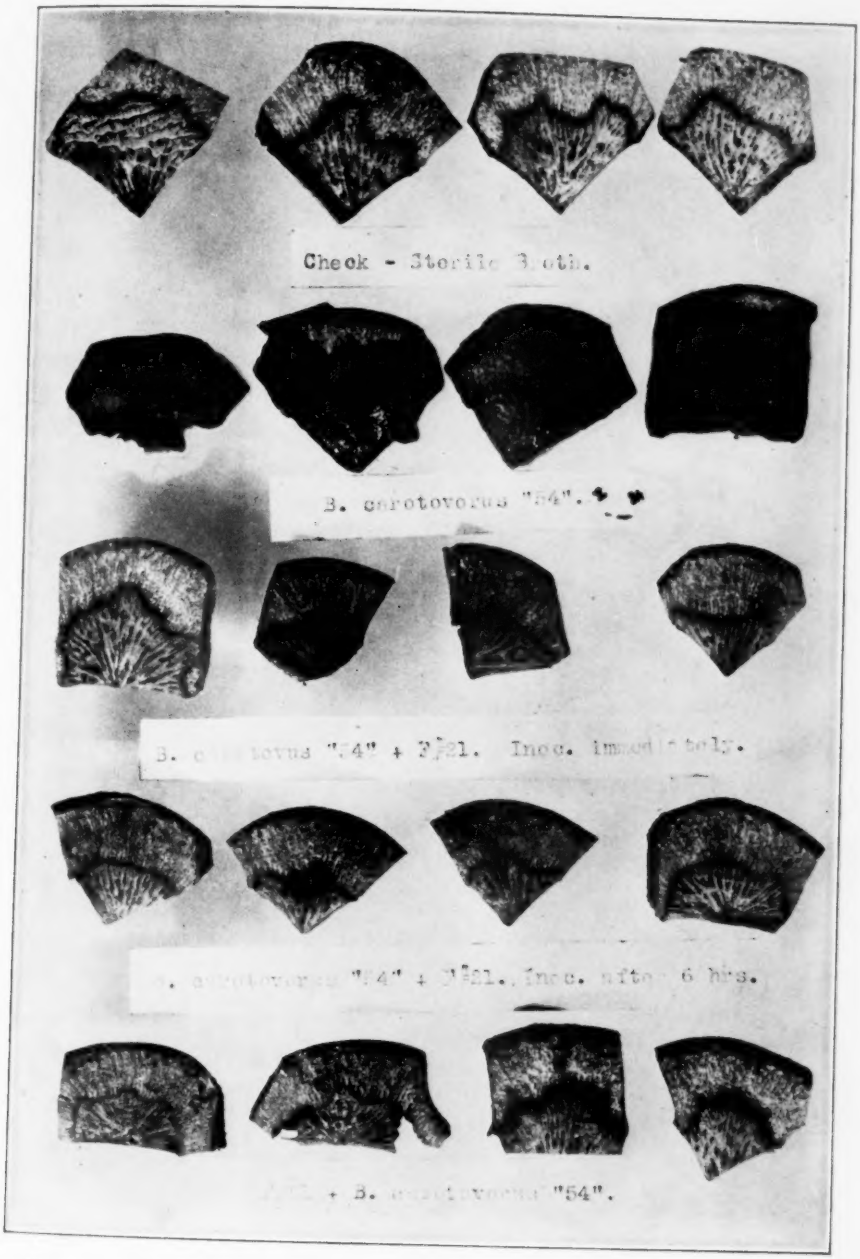

PLATE XI. Protective effect of the lytic principle to raw carrot against infection by *Bacillus carotovorus*.

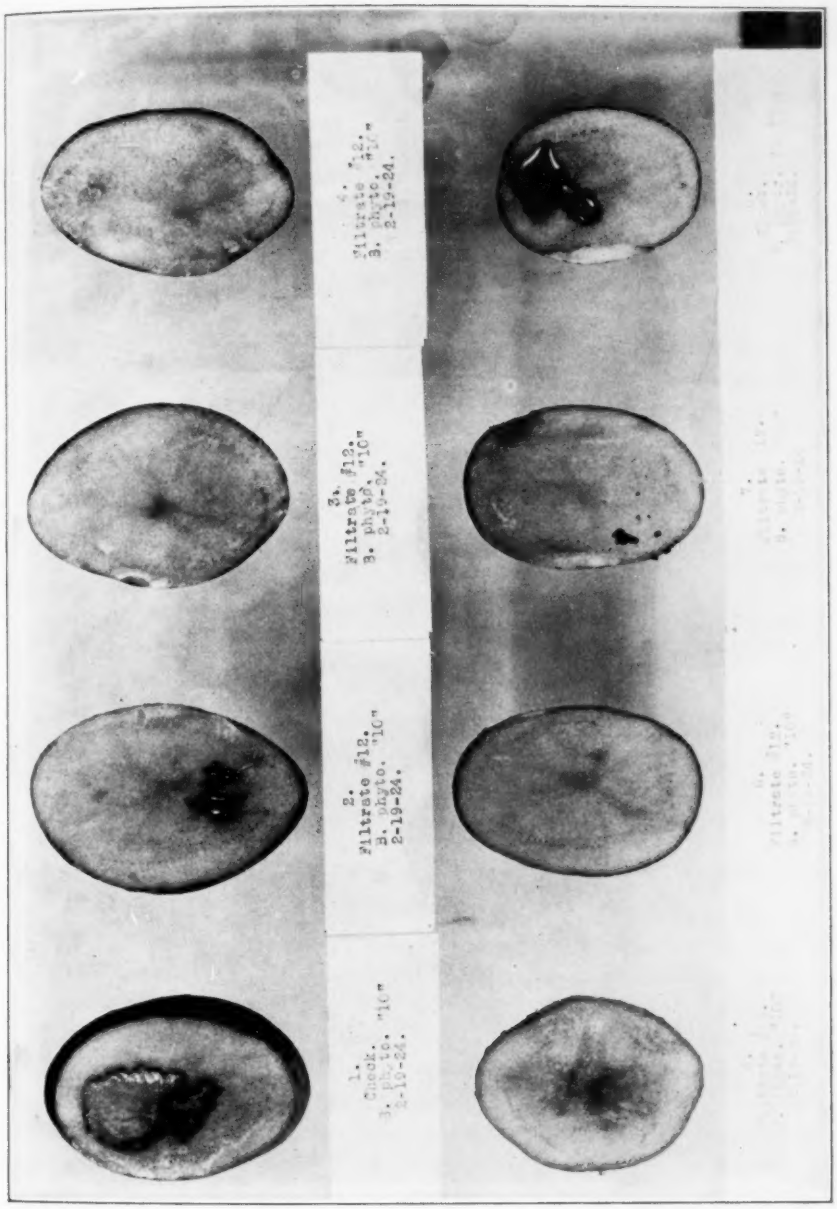

PLATE XII. Protective effect of the lytic principle to raw potato slices against infection by *Bacillus atrosepticus*.

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INHERITANCE IN WHEAT OF RESISTANCE TO BLACK STEM RUST[1]

H. K. Hayes, E. C. Stakman, and O. S. Aamodt

With Plate XIII

INTRODUCTION

The problem of developing varieties of wheat which are resistant to stem rust (*Puccinia graminis tritici* Erikss. and Henn.) is complex. Previous studies on the mode of inheritance of resistance and on the pathogenicity of the causal organism have placed the problem on a definite research basis. The present study was made on the inheritance of resistance in a cross between parental material in which there were two types of resistance.

A BRIEF REVIEW OF PREVIOUS STUDIES

It is now known that *Puccinia graminis tritici* consists of forty or more parasitic strains or physiologic forms (10, 11, 15, 16, 17). These physiologic forms can be distinguished by their action in the greenhouse on seedlings of twelve wheat varieties belonging to five groups (17). The following key was devised by Stakman and Levine (17) to indicate the types of reaction of the differential hosts to physiologic forms (Table 1).

Plus and minus signs also are used to indicate fluctuations within a given type. Thus, 3 — indicates that the reaction falls in the 3 class but that it is fairly weak for that class. On the other hand, 3 + indicates that the type of infection is 3 but that it is near the upper limit of the 3 class.

The type of infection on seedlings in the greenhouse is not always the same as that which develops on the same variety in the field. It is difficult to make studies of the reaction of varieties to single rust forms in the field because other forms than the one in question may infect the plants. However, in some seasons when rust was not very prevalent, it was possible to determine the reaction of varieties to particular physiologic forms in the field (5, 6). By correlating the reaction of varieties to several physiologic forms in the greenhouse with the reaction under epidemic conditions in the field, it has been possible to obtain certain information regarding the rela-

[1] Cooperative investigations between the Sections of Plant Breeding and Plant Pathology of the Minnesota Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

Published with the approval of the Director of the Minnesota Agricultural Experiment Station as Paper No. 518 of the Journal Series of the Minnesota station.

TABLE 1.—*Explanation of symbols used to indicate types of infection produced by physiologic forms of Puccinia graminis on varieties of wheat*

0—IMMUNE

No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

1—VERY RESISTANT

Uredinia minute and isolated, surrounded by sharp, continuous, hypersensitive, necrotic areas.

2—MODERATELY RESISTANT

Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green but slightly chlorotic islands.

3—MODERATELY SUSCEPTIBLE

Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4—VERY SUSCEPTIBLE

Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

X—HETEROGENEOUS

Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill-defined.

tionship of the reaction of seedlings in the greenhouse to that of older plants in the field.[3]

Stakman and Levine (17) considered that varieties which develop types of infection 0, 1, and 2 in the greenhouses are resistant; while those which develop types 3 and 4 are susceptible. For practical purposes it probably would be preferable to designate plants which develop a 3-type reaction as moderately resistant. It is known definitely that varieties and hybrids on which 0, 1, and 2 types of infection occur in the greenhouse also are resistant to the same forms of rust in the field. But it also is known that seedling plants, apparently susceptible in the greenhouse, are sometimes rather highly resistant in the field. This applies particularly to the 3, 4, and X types of reaction. Studies of the nature of resistance to *Puccinia graminis* may explain these apparent discrepancies.

It is known definitely that there are two types of resistance: (1) a true protoplasmic resistance which varies relatively little, and (2) a morphological resistance which varies with the age of the host and the conditions under which it has grown. Stakman (14) showed that resistance to physiologic forms of *Puccinia graminis* often is due to a real physiological incom-

[3] Harrington, J. B. The inheritance of resistance to *Puccinia graminis tritici* in crosses between varieties of durum wheat. (In press).

Levine, M. N. Statistical studies on the variation of physiologic forms of *Puccinia graminis tritici* and the effects of ecological factors on the susceptibility of wheat varieties. 1924. [Unpublished thesis for degree of Doctor of Philosophy, Univ. Minn.]

patibility between the resistant plants and the invading fungus. He showed that when a germ tube entered a resistant host it apparently secreted some toxic substance which quickly killed some of the cells of the inoculated plant. The fungus itself then died, probably either because of its inability to obtain nutrients from the dead cells or because of the liberation of a harmful substance by the host cells. It was shown that the struggle between host and parasite was short and decisive and involved only a few cells in the most resistant plants. In plants which are only moderately resistant, on the other hand, the rust hyphae do not kill so quickly. Larger areas are involved, which appear as necrotic lesions to the unaided eye, and the rust hyphae are able to live for some time. Uredinia may even be formed, but the development of the rust is distinctly subnormal. In susceptible varieties, however, the fungus apparently does not injure the host cells immediately but actually seems to stimulate them to increased physiological activity. This often results in the production of green islands.

Allen (2, 3) confirmed these conclusions and suggested also that many uredinial germ tubes apparently were unable to enter resistant hosts. In other words, certain resistant varieties had two methods of defense against the pathogene: (1) true protoplasmic resistance as described above, and (2) some morphological peculiarity such as size of stomata or some physiological characteristic which made it difficult for germ tubes to enter.

Hursh (8) has proved rather conclusively that wheat varieties may be resistant on account of morphological peculiarities. It is known that the rust mycelium develops almost exclusively in the chlorenchymatous collenchyma of wheat stems. In some varieties the collenchyma bundles are small and are separated by sclerenchymatous fibers. There is therefore a definite mechanical limitation to the spread of the rust mycelium. As the amount of collenchyma is inversely proportional to the amount of sclerenchyma, varieties with a large amount of sclerenchyma would be resistant. There may be no real protoplasmic resistance, but pustules are likely to be small and linear because the areas of host tissue in which the rust can develop are narrow. The amount of sclerenchyma in proportion to collenchyma increases as plants grow older. This, then, would account for the fact that seedlings sometimes may appear to be susceptible in the greenhouse, but older plants of the same variety may be resistant in the field.

Previous studies of crosses in which the two parents react reciprocally to two physiologic forms of rust have demonstrated in all cases that it is possible to obtain homozygous hybrids which are resistant to both physiologic forms, others susceptible to both forms, as well as others which react in the same manner as the parent varieties (4, 6, 7, 12).

In the present study one parent was immune from certain physiologic forms in the greenhouse, and the other was susceptible in the greenhouse

but rather resistant to many forms of rust in the field. The parent varieties were selections from previous crosses. As one of the parents was obtained from a durum-vulgare cross, it appears desirable to discuss the problem of species crosses in relation to rust resistance.

PROBLEM OF SPECIES CROSSES IN WHEAT

Sax (13), Watkins (18), and Kihara (9) have recently carefully studied the problem of wheat species crosses from the cytologic and genetic standpoints. From the standpoint of rust resistance, wheat breeders are chiefly interested in crosses between parent varieties belonging to the emmer and vulgare groups, which contain 28 and 42 chromosomes, respectively. The studies referred to have proved rather conclusively that there is a rapid return to the parental or species chromosome numbers in generations following a cross when varieties belonging to the emmer and vulgare groups are used as parents.

Sterility in generations following the cross has been attributed by Sax to unbalanced numerical relations of the chromosomes, due to random distribution of the univalents and to incompatibility of certain combinations among the bivalents. Of particular interest in relation to rust resistance is the study of Sax in which correlations of chromosome number, morphological characters, and rust resistance were made in the F_3 generation. For the relation between chromosome number and rust resistance, only 37 plants were available. It is very evident from the results presented that the correlation between the 14-chromosome group and rust resistance and between the 21-chromosome group and rust susceptibility was rather close in this study. Of the 12 plants classed as common types and belonging to the 21-chromosome group, one showed considerable resistance, although it was not so resistant as the Kubanka parent. From these studies it is clear that it is difficult to combine the rust resistance of the durum parent with the chromosome number of the vulgare parent when relatively few individuals are available. Sax further concludes that "The characters peculiar to the common wheats are dependent on the assortment of the 7 univalent chromosomes and as a result these characters, in a homozygous condition, are, with few exceptions, found only in segregates with 21 chromosomes." Sax discusses a study made in Minnesota (5) in which over 20,000 F_3 segregates from a cross between rust-resistant durums and common wheats were examined. He emphasizes the important fact that only a few plants were found with the morphological characters of the common wheat parent which were also resistant to rust. In view of the importance of the problem of combining the rust resistance of a durum parent and the desirable characters of the common parent, a brief review of results to date will be of interest.

One of the parents of the cross to be discussed later in this paper is Marquis × Iumillo, N. S. N. II–15–114. This new variety bears the Minnesota Number 2202 and the Cereal Investigations Number 6887. It is the most promising selection obtained from the cross between Iumillo, a red-seeded durum, and Marquis. From unpublished studies made by Dr. Fred Griffee, of the plant breeding section, this hybrid variety is known to have the chromosome number of vulgare wheats. It has been studied rather widely under field conditions and has proved highly rust resistant, although it is not as resistant as some of the better selections of Iumillo. Unfortunately, in the original Iumillo × Marquis cross, the Iumillo parent was not pure, and a reported study (5) of infection of the Iumillo parent showed that the hybrid was as resistant in the greenhouse to physiologic form 1 as the Iumillo parent (C. I. 1736). Numerous selections have been made in Iumillo and some are now available which are much more resistant than the original Iumillo variety.

The difficulties of wheat-species crosses have been emphasized. However, it seems apparent that the possibilities are unknown. The Marquis-Iumillo hybrid II–15–44 is, perhaps, the most promising selection yet obtained from a wheat-species cross. For this reason a summary statement of its performance in relation to Marquis is interesting. Data were obtained on comparative rust reaction in the rust epidemic nursery. The epidemic was induced by the use of all available physiologic forms of *P. graminis tritici*. The Marquis × Iumillo hybrid appears rather highly resistant (see table 2).

TABLE 2.—*Rust reaction of Marquis, Iumillo, and Marquis × Iumillo II–15–44*

Year	Physiologic forms available	Percentage rust infection		
		Marquis	Marquis × Iumillo	Iumillo
1920	1, 3, 9	90	5	0
1921	1, 3, 9, 14, 17, 18, 19, 21, 29, 32	50	2	0
1923	1, 3, 9, 11, 17, 18, 19, 21, 34	70 S	15 R & SR	15 R
1924	1, 3, 9, 17, 18, 19, 21	50 S	Tr	0

The method of indicating rust reaction in percentage of infection has been described previously. In recent years, in the rust nursery, the letters, S, SR, and R also have been added. These refer to the type of infection. Very small uredinia, as well as long linear ones are considered to indicate resistance (R). An intermediate type which appears less well developed than the susceptible group is designated as semi-resistant (SR) while the presence of normal, large, healthy uredinia indicates complete susceptibility (S).

Data on yielding ability and rust reaction are given in table 3. Apparently the hybrid variety is rather highly resistant under field conditions in many localities. At University Farm, in 1924, there was a very serious infection with root-rot. The durum varieties are particularly susceptible. While not so severely injured as durum wheats the new hybrid, II–15–44, was more severely injured than Marquis.

The hybrid was rather highly resistant in the various field studies, and in years when rust is destructive it yields much better than Marquis.

Preliminary milling and baking trials of the hybrid and of Marquis have been made under the direction of Dr. C. H. Bailey, of the Department of Agricultural Biochemistry, University of Minnesota. The seed for the milling trials was obtained from rod-row plots. In 1922 seed was available from University Farm only, while in 1923 comparative milling tests were made of University Farm material and also of a mixture of equal portions of seed obtained from the Morris, Crookston, and Waseca branch stations. The seed of Marquis grown at University Farm was fairly plump while that from the branch stations was severely injured by black stem rust.

TABLE 4.—*Milling and baking results of Marquis and Marquis × Iumillo II–15–44*

Variety or Hybrid	Year grown	Place grown	Flour						Plumpness of grain
			Protein	Loaf volume	Water used	Texture score	Color score	Total flour	
			pct.	cc.	cc.			pct.	pct.
Marquis × Iumillo 11–15–44	1922	U. Farm	13.05	1540	58.1	98	99	75.8	81
Do	1923	U. Farm	16.73	2110	56.4	97	96	67.8	84
Do	1923	C. M. W.	16.25	1940	57.7	96	95	73.6	79
Average			15.34	1863	57.4	97.0	96.7	72.4	82
Marquis	1922	U. Farm	12.03	1590	59.9	99	99	74.0	80
Do	1923	U. Farm	16.16	2000	57.8	99.5	99.5	68.6	79
Do	1923	C. M. W.	15.65	1860	57.3	94	97	70.7	39
Average			14.61	1817	58.3	97.5	98.5	71.1	66

A comparison of the baking tests indicates that the hybrid is nearly the equal of Marquis in baking quality. Probably the hybrid is slightly inferior to Marquis in its color score. The results justify the conclusion that it is possible to combine in a common wheat the rust resistance obtained from a durum wheat parent and the milling quality of the common wheat parent. It is true that the hybrid appears slightly inferior to Marquis but the differences are very small.

Another series of crosses was made in 1920 between Mindum, a variety of *Triticum durum*, and Marquis and Velvet Chaff, two varieties of *T. vul-*

TABLE 3.—*Comparative yielding ability and percentage of rust infection of Marquis and Marquis × Iumillo II–15–44**

Type of plot	Locality	Year of test	Yield, bushels per acre		Percentage rust infection	
			Marquis	Marquis × Iumillo	Marquis	Marquis × Iumillo
Rod rows	Univ. Farm, St. Paul, Minn.	1921	18.4	16.1	0	0
"	" " " " "	1922	36.0	40.0	41	T
"	" " " " "	1923	31.0	32.4	22	T
"	" " " " "	1924	26.9	22.7	0	0
"	Waseca, Minn.	1922	19.7	29.1	35	3
"	" "	1923	22.2	22.6	23	4
"	Crookston, Minn.	1922	28.1	26.8	20	2
"	" "	1923	15.8	21.8	58	T
"	Morris, "	1922	24.8	27.2	24	0
"	" "	1923	16.4	21.8	88	15
"	Ave. of 6 localities, Canada	1923	28.2	37.4	30–75	5–15
"	" " " " "	1924	28.8	26.2	0–45	0–3
"	Mandan, N. D.	1924	21.8	22.6	24	3
"	Dickinson, N. D.	1923	11.2	15.7	50	20
"	" " "	1924	20.9	11.8†	25	5
"	Moccasin, Mont.	1923	36.6	37.7	0	0
"	" "	1924	36.9	32.4	0	0
"	Fargo, N. D.	1924	38.4	37.0	64	2
"	North Platte, Neb.	1924	31.4	25.1	0	0
1/40 Acre	Crookston, Minn.	1924	20.5	30.0	95	50
"	Morris "	1924	28.9	27.9	42	1
"	Waseca "	1924	37.2	42.1	80	1
"	Grand Rapids, Minn.	1924	15.0	15.4	87	42
"	Univ. Farm, St. Paul, Minn.	1924	33.3	32.0	0	0
"	Moccasin, Mont.	1924	32.0	31.5	0	0
"	Brandon, Man.	1924	44.0	55.3	70	20

* Acknowledgement is made to various investigators who have kindly furnished comparative data which they have taken. Through such cooperation a comparison under many different conditions has been made possible. The data obtained from 6 places in Canada were furnished by Prof. W. P. Fraser; Mr. J. A. Clark, of the U. S. Dept. of Agriculture, furnished data from Mandan and Dickinson, North Dakota, and Moccasin, Montana; Professor L. R. Waldron furnished the data from Fargo; Mr. S. J. Sigfusson furnished the report from Brandon, Manitoba, Canada.

The data collected at the branch stations in Minnesota were obtained from Prof. A. C. Arny for the 40th-acre plot tests. The various branch station agronomists, R. E. Hodgson, Waseca; R. O. Bridgford, Morris; R. S. Dunham, Crookston; O. I. Bergh, Grand Rapids; and M. J. Thompson, Duluth, assisted in the collecting of data obtained from the branch stations.

† The low yield was probably a result of root-rot.

gare. It was hoped to make a careful study of fruitfulness of the F_2 hybrids in relation to plant type, and, in order to prevent cross pollination, spikes of each plant were covered with a glassine bag. The season of 1921 was not very favorable and for this reason the results have not been studied in detail. It also was learned that the degree of fruitfulness of Mindum was much more seriously affected by covering with a paper bag than that of Marquis and this interfered with a careful genetic analysis of the data. As in the previous study, the various plants were placed in seven general groups, largely on the basis of keel development, *viz*: durum, near-durum, intermediate, near-common, common, emmer-like, and emmer. The plants of the two groups, common and near-common, were selected and their progeny grown in F_3. Forty-four lines were available. Fourteen lines which appeared promising, and which appeared to be breeding true for the vulgare habit, were harvested, several plants from each line being chosen. These were threshed individually and the reaction of their F_4 progeny was tested in the greenhouse to two physiologic forms of rust to which Mindum was resistant and Marquis susceptible. The results of this trial are presented in table 5.

TABLE 5.—*Reaction of Marquis, Mindum, and fourteen F_4 lines of Marquis × Mindum or Marquis × Velvet Chaff to two physiologic forms to which Marquis is susceptible and Mindum resistant*

Parents or crosses	Types of reaction to physiologic forms	
	1	18
Mindum	Flecks and 1	Flecks and 1
Marquis	4	4
Marquis or Velvet Chaff × Mindum, F_4, 12 families	All produced some 4's. A few families were heterozygous, the remainder produced only susceptible plants.	All susceptible
Velvet Chaff × Mindum, F_4, No. 70	Flecks, 1, and a few 3	Mostly 4
Marquis × Mindum, F_4, No. 25	1 and 2	1

All investigators who have studied species crosses agree that vulgare types, as a rule, can be determined by inspection, although further studies of spike morphology and cytological condition should be made. The seedlings of one family of vulgare appearance were resistant to both physiologic forms 1 and 18 in the greenhouse. Another family was susceptible to form 18 and resistant to form 1. The Velvet Chaff parent was not tested. The remaining 12 families were homozygous for susceptibility to form 18 and either heterozygous or susceptible in their reaction to form 1. These data

furnish further proof of linkage between the vulgare characters and susceptibility in crosses between resistant durums and susceptible commons. They indicate that the linkage is not absolute and furnish a further reason for the hope that rust-resistant common wheat can be obtained from crosses between susceptible varieties of *T. vulgare* and resistant varieties of *T. durum*.

Besides presenting data in relation to the possibilities from wheat species crosses, the purpose of the present paper is to describe the mode of inheritance of rust resistance in a cross in which the parents were known to have two different types of rust resistance. It is known that the resistance of the Marquis-Kanred hybrid is protoplasmic. Whether the resistance of the Marquis-Iumillo parent is physiologic or morphologic in nature, or both, is not known definitely.

GENETIC FACTORS INVOLVED IN TWO TYPES OF RUST RESISTANCE

The study of inheritance of resistance was made in the F_2 to F_4 generations of a double cross (Marquis × Iumillo) × (Marquis × Kanred). The Marquis × Iumillo parent is the strain already described under the N. S. N. II–15–44 which, under field conditions, has proved rather resistant to various physiologic forms of *P. graminis tritici*. It obtained this resistance from the durum variety, Iumillo. Two different Marquis × Kanred hybrid lines were used as parents. Both reacted under greenhouse conditions in the same manner as their Kanred parent. From previous studies they are considered to be immune from eleven of the twenty-one physiologic forms found in the north-central spring wheat section (1). The purpose of the study was to learn the factors of resistance involved and the possibilities of combining in one variety the resistance of both parents. The problem was one phase of an attempt to produce desirable spring wheat varieties resistant to black stem rust. Although the Marquis × Iumillo parent has been rather resistant under field conditions in all tests so far made, it shows little or no resistance in the seedling stage to various physiologic forms of rust when the study is made under greenhouse conditions. Seedlings of the two Marquis × Kanred parents are both immune, in the greenhouse, from the physiologic forms from which Kanred is immune. As both of the Kanred × Marquis strains used as parents reacted in a similar manner, and as their progeny segregated in a similar manner in the crosses with Marquis × Iumillo, the results have been combined in the tables.

The study of inheritance was carried on with approximately 250 F_3 lines from the double cross (Marquis × Iumillo) × (Marquis × Kanred). These F_3 lines represented a random sample of F_2 plants not previously studied for their rust reaction. It is obvious that the most accurate means

of determining the genotypic condition of F_2 plants with respect to disease resistance or susceptibility is to study the breeding behavior of F_3 lines.

In previous studies it was learned that the immunity of Kanred was a dominant character and that immunity from all physiologic forms from which Kanred was immune was dependent on a single genetic factor. The study of inheritance of the Kanred type of immunity in the double cross (Kanred × Marquis) × (Marquis × Iumillo) was made by growing from 20 to 40 seedlings of each F_3 hybrid line in the greenhouse and inoculating them with spores of form 21. As in previous studies, a single genetic factor pair differentiated immunity and susceptibility and the F_3 lines were placed in three groups: I, homozygous for immunity, producing types of infection only in the 0 class; H, heterozygous, producing both 0 and 4 types of infection with a ratio of approximately 3 immune to 1 susceptible; and S, producing only susceptible types of infection in the 3 and 4 classes.

The same hybrid lines were grown under field conditions and an artificial epidemic was induced by inoculating with forms 1, 3, 9, 17, 18, 19, 21, and 34. Physiologic form 11 was obtained from a culture taken from the rust nursery and therefore was present as a result of natural infection. The heavy epidemic in 1923 made it possible to differentiate clearly between resistance and susceptibility. However, there often is no sharp line between resistance and susceptibility under field conditions. The various families from which approximately 25 seeds were sown were studied by the individual-plant method, the plants being placed in three groups according to type and extent of infection as follows:

R, Resistant, with only a relatively small amount of injury from rust. The uredinia produced are small or of the narrow-linear type.

SR, Semi-resistant, with uredinia of an intermediate type of development.

S, Susceptible, with normal, well-developed uredinia.

Rows of Marquis wheat were grown throughout the series and were similarly studied. This variety was completely susceptible throughout.

The Kanred-Marquis parent was somewhat resistant under field conditions. This resistance was not due to the immunity from certain physiologic forms but to a tendency to resistance characteristic of some Kanred hybrids. The resistance, however, can not be studied accurately as it is not striking enough to be differentiated from fluctuations due to environmental conditions. Under field conditions the hybrids and parent lines were placed in groups according to their reactions. In most cases enough plants were available to give an accurate indication of the genotypic con-

dition. Susceptibility proved to be dominant and therefore the classification of resistant lines was probably approximately correct.

Some idea of the accuracy of the investigation can be gained by a study of the behavior of the parent cultures which were distributed throughout the greenhouse and field and which were studied in the same manner as the hybrids. Under greenhouse conditions, the parents and hybrids were classed as homozygous for immunity (I); heterozygous, with immunity dominant (H); and susceptible or homozygous for susceptibility (S).

The F_3 lines grown in the field were studied by the individual plant method. The Marquis × Iumillo, II–15–44, parent was resistant, although approximately half of the plants were more severely infected than the others. None, however, was as susceptible as Marquis. About half of the plants in each row of the Marquis × Iumillo parent were classed as SR and the remainder as R. This was considered as the expected phenotypic expression of the factors for resistance of the Marquis × Iumillo parent. Classes for field reaction were as follows:

R, Resistant or with approximately half of the plants R and the remainder SR, and with no S plants.
H, Heterozygous, with a small percentage of plants in the R group, the greater proportion being classed as SR or S.
In, Intermediate, or all plants with the SR type of infection.
S', Containing both SR and S types of infection.
S, Susceptible, containing only S types of infection.

The classification of the reaction of the parent lines under greenhouse and field conditions is given in table 6.

TABLE 6.—*Classification of parent lines, Marquis × Iumillo and Marquis × Kanred, on the basis of their reaction to stem rust under greenhouse and field conditions*

Parent	No. of separate tests	Reaction to physiologic form 21, in the greenhouse	Field reaction
Marquis × Iumillo	8	S	R
" "	1	S	H
" "	2	H	R
" "	2	I	In
Total	13		
Marquis × Kanred	5	I	S'
" "	6	I	In
" "	1	I	H
" "	1	H	S'
" "	1	H	In
Total	14		

The four cultures of Marquis × Iumillo, classed as H or I on the basis of the type of infection in the greenhouse, probably escaped infection. The 0 type of infection predominated on the two lines of the Marquis × Kanred parent which were classed as H. Of the Marquis × Iumillo parent lines under field conditions, 10 out of 13 were classed as resistant. This shows the degree of accuracy with which it was possible to classify the various lines under the conditions of the experiment. Experience in studies where physiologic forms are used leads to the belief that the results may be accepted in general as accurate indications of the nature of the genetic factors involved. In order to be absolutely sure of a particular classification from the breeding standpoint, a particular line must be studied through several generations.

The various hybrid families are classed according to greenhouse and field types of infection by the method already described. The results of this study are given in table 7.

TABLE 7.—*Classification of 249 F_3 families of the cross of (Marquis × Iumillo) × (Marquis × Kanred) on the basis of the reaction of seedlings to physiologic form 21 in the greenhouse and of older plants under field conditions to an epidemic of stem rust produced artificially by several physiologic forms*

	Number of F_3	Classification for greenhouse reaction	Classification for field reaction
	6 + 1*	I	R
	19	I	H
	8 + 1	I	In
	15 + 2	I	S[1]
	3	I	S
Total	51 + 4		
	10 + 1	H	R
	45 + 2	H	H
	15 + 1	H	In
	55 + 4	H	S[1]
	6	H	S
Total	131 + 8		
	0 + 2	S	R
	7	S	H
	3	S	In
	32 + 2	S	S[1]
	9	S	S
Total	51 + 4		

* The families classed as + 1, etc., are of doubtful nature chiefly because of small numbers involved.

It is apparent that only a single factor pair is involved for reaction to physiologic form 21 under greenhouse conditions. Under field condi-

tions, 16 families with a +4 reaction in the greenhouse out of a total of 249 are classed as resistant, with the type of resistance of the Marquis × Iumillo parent. This ratio agrees fairly well with a 15:1 ratio and indicates that the Marquis × Iumillo type of field resistance is dependent on the interaction of two genetic factors, both of which must be present in a homozygous condition to produce resistance. The allelomorphs of these factors, either alone or in combination and apparently in either the homozygous or heterozygous condition, lead to susceptibility.

Besides the F_3 generation lines which were grown in 1923, F_2 families in which Marquis × Iumillo was one parent have been studied under rust conditions. Most of the plants have been susceptible, although some resistant plants appear in such F_2 progenies. These facts furnish further proof that more than a single genetic factor determines resistance of the Marquis × Iumillo parent in the field.

The genetic factors for resistence of the Marquis × Iumillo parent under field conditions are inherited apparently independently from the factor for immunity from certain physiologic forms under greenhouse conditions, although the nature of the material is such that a loose linkage could not be demonstrated. At any rate, it is apparent that any combination of the parental types of resistance or susceptibility can easily be obtained in the progeny.

One F_3 family appeared more resistant than others. Desirable plants from resistant families and resistant plants obtained from susceptible families were selected and their F_4 progeny were grown in 1924. With the exception of one or two lines, the 1924 progeny appeared rather highly resistant, although some lines appeared more resistant than others. The resistance appeared much greater than in previous tests of F_4 Kota × Marquis crosses, indicating that perhaps fewer genetic factors were involved in the resistance of Marquis × Iumillo than for Kota wheat. One F_3 line, the most resistant of all, was tested in F_4 and again proved more resistant than the other lines.

While there is an indication of two main genetic factors, modifying factors also apparently are involved.

DISCUSSION OF RESULTS

The discovery of physiologic forms of stem rust aided materially in placing the breeding of rust-resistant varieties on a definite genetic basis. The problem is obviously one of combining in a single variety of wheat the resistance of particular wheat varieties by appropriate crosses and recrosses.

While some common wheats of the *T. vulgare* group are resistant to certain physiologic forms under field conditions, it is apparent that varieties

of *T. dicoccum* and *T. durum*, of the emmer group, are most resistant. The Marquis × Iumillo hybrid used as one parent in the present study obtained its resistance from the durum parent, Iumillo. From crosses between Mindum, a durum variety, and Marquis and Velvet Chaff, 14 F_3 families were obtained which appeared to be homozygous common types. This classification was made on the basis of keel and spike characters, although the keel was the main means of classification. F_4 progeny of these families were tested in the greenhouse to two physiologic forms of rust to which Mindum was resistant and Marquis susceptible. One family, which was tested by studying the progeny of three plants, was resistant to both physiologic forms of rust. This family was the result of a cross between Marquis and Mindum. One family of Velvet Chaff × Mindum was resistant to one physiologic form and susceptible to the other, while the remaining families were predominantly susceptible, although occasional plants were less severely infected than others.

These results indicate that it is possible to transfer rust resistance from the 14-chromosome group to the 21-chromosome group. That no commercial varieties have yet been obtained from such crosses perhaps may be due to the large number of factors involved as well as to chromosome behavior. The genetic difficulties are so great that one can hardly expect to obtain a segregate which contains all desired characters, especially when the parents differ in chromosome number and many characters of economic importance. It has been demonstrated by these studies, however, that rust resistance can be transferred from the 14-chromosome group to the 21-chromosome group.

In the crosses of Marquis × Iumillo with Marquis × Kanred there apparently were two factors involved for the Marquis × Iumillo type of resistance. On the chromosome basis of heredity it seems logical to conclude that at least two chromosomes or parts of chromosomes which contained factors for rust resistance were obtained from the Iumillo durum parent and that these were combined with chromosomes of the common group, which led to the production of a rust-resistant common wheat with 21 chromosome pairs.

Because of the obvious importance of the problem it is hoped that more intensive studies of the genetic and cytologic phases of wheat-species crosses may be made in the future. In the meantime the results reported give further hope that it may be possible to combine in a 21-chromosome wheat the important characters of bread wheats together with certain desirable durum characters such as rust resistance.

SUMMARY

1. In order to obtain wheat varieties resistant to all physiologic forms of stem rust it is necessary to make crosses and recrosses. The purpose of such crosses is to obtain a desirable variety of *T. vulgare* resistant to all physiologic forms prevalent in the area growing spring wheats.

2. Certain durum and emmer wheats are more generally resistant to the physiologic forms than any known varieties of the common group. The Marquis × Iumillo hybrid, II–15–44, was obtained from a cross of Iumillo durum and Marquis. So far as tested, it is rather rust resistant under field conditions, yields well, and is not apparently greatly inferior to Marquis in milling quality, although more milling and baking tests are needed before final conclusions should be drawn.

3. The importance of a knowledge of the possibilities of combining in a variety of *T. vulgare* the desirable qualities of bread wheats, together with such characters as rust and drouth resistance which can be obtained from durum varieties, emphasizes the necessity of further intensive and extensive genetic and cytologic studies of wheat-species crosses.

In a cross of the common wheats, Marquis and Velvet Chaff with Mindum, a durum wheat, fourteen F_3 families of apparent vulgare habit were selected. The reaction of the F_4 progeny of these 14 families to two physiologic forms to which Mindum was resistant and Marquis was susceptible was determined under greenhouse conditions. One family of the Marquis × Mindum cross, resistant to both physiologic forms, was obtained. This resistance was derived from its durum parent. These facts, together with the Marquis × Iumillo hybrids, prove the possibility of obtaining rust resistance from a durum parent and combining it with the chromosome number of common wheats.

4. In a double cross, (Marquis × Iumillo) × (Marquis × Kanred), the inheritance of two types of resistance was studied. The Marquis × Iumillo parent is resistant to many physiologic forms of stem rust under field conditions, but it is susceptible in the seedling stage in the greenhouse. The Marquis × Kanred parent is immune from 11 of the 21 physiologic forms found in the hard red spring wheat region both in the greenhouse and under field conditions. The Marquis × Kanred type of immunity is dependent upon a single factor pair, while at least two factors are necessary to explain the resistance of the Marquis × Iumillo parent. The factors for resistance of the Marquis × Iumillo parent apparently are inherited independently of the factor for immunity of the Marquis × Kanred parent.

5. All combinations of resistance and susceptibility of the parents were obtained in the hybrids. Homozygous types were obtained which con-

tained the factors for resistance from both parents, as well as homozygous types which were susceptible both under greenhouse and field conditions.

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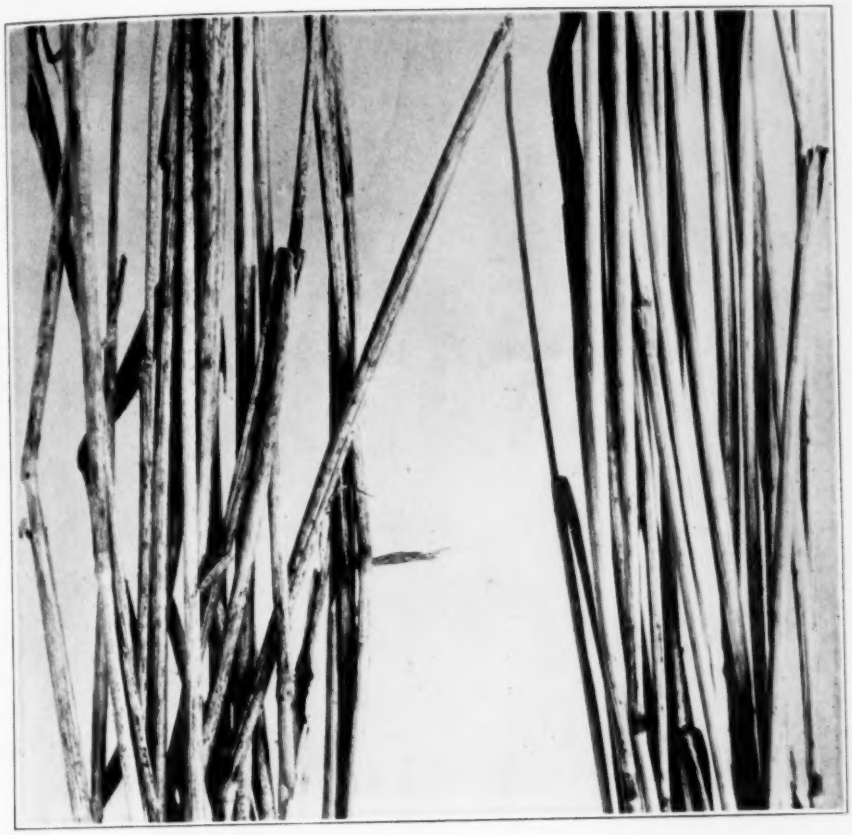
LITERATURE CITED

1. AAMODT, O. S. The inheritance of growth habit and resistance to stem rust in a cross between two varieties of common wheat. Jour. Agr. Res. **24**: 457–470. 1923.
2. ALLEN, R. F. A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici*. Jour. Agr. Res. **23**: 131–152. 1923.
3. ———. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms 3 and 19. Jour. Agr. Res. **26**: 571–604. 1924.
4. HARRINGTON, J. B., and O. S. AAMODT. The mode of inheritance of resistance to *Puccinia graminis* with relation to seed color in crosses between varieties of durum wheat. Jour. Agr. Res. **24**: 979–996. 1923.
5. HAYES, H. K., J. H. PARKER, and C. KURTZWEIL. Genetics of rust resistance in crosses of varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum*. Jour. Agr. Res. **19**: 523–542. 1920.
6. ———, and E. C. STAKMAN. Wheat stem rust from the standpoint of plant breeding. Proc. Ann. Meeting West. Canad. Soc. Agron. (1921) **2**: 22–35. 1922.
7. ———, and O. S. AAMODT. A study of rust resistance in a cross between Marquis and Kota wheats. Jour. Agr. Res. **24**: 997–1012. 1923.
8. HURSH, C. R. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici* Erikss. and Henn. Jour. Agr. Res. **27**: 381–412. 1924.
9. KIHARA, H. Cytologische und genetische Studien bei wichitigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität der Bastarden. Mem. Col. Sci. Kyoto Imp. Univ. (B) No. 1, Art. 1. 1924.
10. LEVINE, M. N., and E. C. STAKMAN. A third biologic form of *Puccinia graminis* on wheat. Jour. Agr. Res. **13**: 651–654. 1918.
11. MELCHERS, L. E., and J. H. PARKER. Another strain of *Puccinia graminis*. Kans. Agr. Exp. Sta. Circ. 68. 1918.
12. PUTTICK, G. F. The reaction of the F_2 generation of a cross between a common and a durum wheat to two biologic forms of *Puccinia graminis*. Phytopath. **11**: 205–213. 1921.
13. SAX, K. The relation between chromosome number, morphological characters, and rust resistance in segregates of partially sterile wheat hybrids. Genetics **8**: 301–321. 1923.
14. STAKMAN, E. C. Relation between *Puccinia graminis* and plants highly resistant to its attack. Jour. Agr. Res. **4**: 193–200. 1915.
15. ——— and F. J. PIEMEISEL. A new strain of *Puccinia graminis*. (Abstract) Phytopath. **7**: 73. 1917.
16. ———, M. N. LEVINE, and J. G. LEACH. New biologic forms of *Puccinia graminis*. (Preliminary paper) Jour. Agr. Res. **16**: 103–105. 1919.
17. ——— and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.
18. WATKINS, A. E. Genetic and cytological studies in wheat. I. Jour. Genetics **14**: 129–171. 1924.

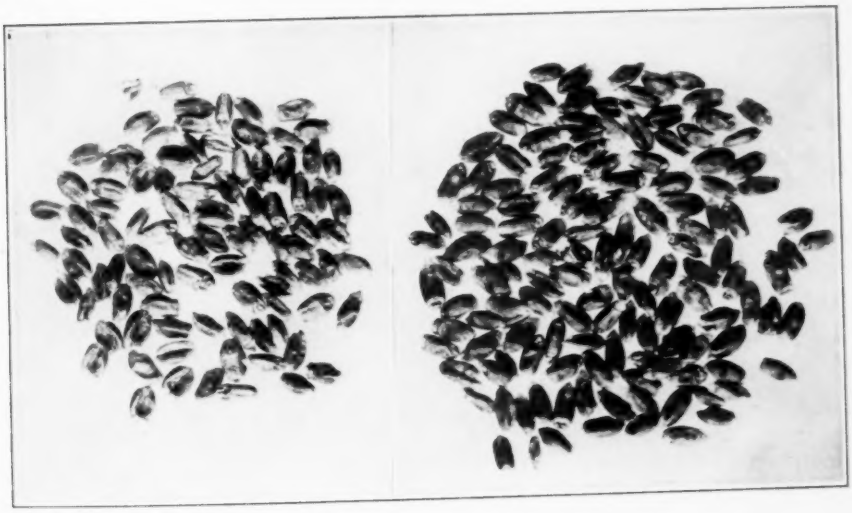
EXPLANATION OF PLATE XIII

Comparison of Marquis and an F_3 hybrid family of the double cross of (Marquis × Kanred) × (Marquis × Iumillo) under artificially induced epidemic conditions in the rust nursery.

A.—Marquis check; B.—An F_3 family which was also highly resistant in F_4; C.—Seed of Marquis check row; D.—Seed of the F_3 hybrid.



A B



C D

FURTHER STUDIES ON PYTHIACEOUS INFECTION OF DECIDUOUS FRUIT TREES IN CALIFORNIA

RALPH E. SMITH AND ELIZABETH H. SMITH

WITH SIX FIGURES IN THE TEXT

A phycomycetous fungus causing a serious rotting of lemons in California was described by the writers (7, 8) in 1906 as a new genus and species, *Pythiacystis citrophthora*. *Pythiacystis* was distinguished from *Pythium* by the formation of swarm spores within the sporangium and the absence of a vesicle, and from *Phytophthora* mainly by the fact that it seemed normally to be a moist soil-inhabiting saphrophyte, rather than an aerial parasite. In the affected tissue of the lemon only sterile mycelium of the fungus was found. Infection originated from zoospores produced in the soil beneath the tree. The statement was made that *Pythiacystis* "forms a close transition from Pythium to Phytophthora" and that it is "more exactly intermediate between the Saprolegnieae and Peronosporeae than Pythium." No oospores or chlamydospores were found. The difference in habitat and parasitic relations between this and typical *Phytophthora*, such as *P. infestans*, seemed sufficient grounds in 1906 for distinguishing *Pythiacystis* from that genus. *Pythiacystis* seemed as close to the Pythiaceae and the Saprolegniales as to *Phytophthora* and the Peronosporales.

The only species of *Phytophthora* in which a soil relation of any significance had been suggested previous to 1906 were *P. omnivora* de Bary (*P. cactorum* (Leb. and Cohn.) Schroet., *Peronospora sempervivi* Schenk, and *Phytophthora fagi* R. Hartig) (1, 2, 3), and *P. nicotianae* Van Breda de Haan (4). In these cases it had been shown that the fungus in the soil may be the source of infection of living plants; but that saprophytic soil or semi-aquatic life is the usual habitat, and that normal sporulation is limited to such conditions were by no means indicated.

Since 1906 a number of new species of *Phytophthora* and new diseases caused by old ones have been described and several have been met with by the writers, the nature of which points strongly toward close relationship between *Pythiacystis* and *Phytophthora*, as the latter genus is now understood, and throws great doubt on the desirability of continuing to regard the former as a distinct genus. Several writers have already suggested such a change (23, 29, 49), but without formally proposing it. Some of these new species also form a close connection between *Pythium* and *Phytophthora* and support the opinion expressed by Fitzpatrick (49) that

Pythium, *Phytophthora*, and *Pythiacystis* should be merged into one genus distinct from the Peronosporaceae (*Peronospora*, *Plasmopara*, etc.) in their absence of well-defined conidiophores and their saprophytic tendencies. Some of the principal species and diseases of this nature which have been described since 1906 are as follows:

Phytophthora cactorum (Leb. and Cohn) Schroet. (*P. omnivora*) (6, 10, 12, 14, 15, 24, 28, 29, 36, 38, 40, 45, 54); *P. nicotianae* Van Breda de Haan (36); *P. syringae* Kleb. (5, 9, 20, 46, 47, 53); *P. faberi* Maub. (11, 33, 52, 54, 56); *P. arecae* (Colem.) Pethy. (13, 20); *P. parasitica* Dastur (16, 50); *P. erythroseptica* Pethy. (19, 34); *P. allii* Sawada (25, 32); *P. melongenae* Sawarda (25, 32); *P. terrestris* Shreb. (31, 35, 39, 41, 43, 48); *P. cryptogea* Pethy. and Laff. (37, 39), and *P. mexicana* Hotson and Hartge (51).

In these cases such effects as rotting of fruit, damping off of seedlings, root rot, stem and twig blight, and similar effects were pronounced. All of the fungi mentioned have been shown to be capable of saprophytic soil life and able to cause infection by contact of plant tissues with the earth or by zoospores produced therein.

In addition to the definite species listed, numerous instances are mentioned in the literature of strains or forms of fungi having similar habits and effects, but differing in morphological and other characteristics from the typical species. In fact, the discovery of such a variety of strains has been the rule rather than the exception in the investigation of many of these Phytophthora diseases. Several different strains or species are commonly found causing apparently the same disease on the same host, and the identification, delimitation, and validity of species are in many cases still considerably in doubt. Oospores have been found in all the species listed except *P. faberi*, in which case chlamydospores are abundant.

LATER KNOWLEDGE OF PYTHIACYSTIS CITROPHTHORA

Faweett (17, 18, 23, 41) showed that this fungus is the cause of a very prevalent type of trunk gummosis of lemon trees in California. Fungi causing foot rot (Mal di Gomma) of orange trees (23, 31, 35, 41) and stem canker of avocado (27, 29, 41) were identified as *P. citrophthora*, but later found to be more typical of *Phytophthora*, having oospores and characters resembling those of *P. terrestris* or *P. cactorum*. Barrett (29) reported the isolation of three strains of a fungus very similar to *Pythiacystis citrophthora*, but with oogonia, oospores, and antheridia similar to those of *Phytophthora cactorum*. True *Pythiacystis citrophthora* isolated from California lemons has been grown in culture by a number of investigators in addition to the writers and Faweett, including Sawada (25).

Barrett (29), and Sherbakoff (31), and found to be distinct from any form of *Phytophthora* studied by them. No sexual bodies and no chlamydospores have ever been found in what may be called typical *P. citrophthora*, but the series of Pythiacystis-Phytophthora-connecting strains discussed in this paper represents a variation which seems very typical of that found by many other investigators.

In 1915 a serious infection of the bark of pear, peach, and almond nursery trees was reported by one of us (26), in which typical *Pythiacystis citrophthora* was found. The fungus produced no spores of any kind in the host tissue and no oospores or chlamydospores in culture. This will be referred to as strain A. There was also obtained from almond trees attacked by the same disease a fungus resembling *Pythiacystis*, but with a somewhat different growth in cultures and production of oospores on agar. This will be designated as strain B.

FIG. 1. Cankers on almond nursery trees from which strains A and B of *Pythiacystis* were isolated.

INOCULATION EXPERIMENTS WITH NURSERY TREES

In the initial experiments, strain B failed to develop when inoculated into pear trees. During the winter of 1916–17 further inoculations were made on nursery trees to test the comparative virulence of the two strains. In these trials, cankers were produced on pear and apple with both strains,

the virulence of the two on all the common fruit trees being about equal. The amount of infection seemed to vary more with the condition than with the kind of tree; succulent, rapidly-growing bark being much more susceptible.

NATURAL INFECTION IN NURSERY TREES FROM 1915 TO 1920

From 1915 to 1920, occasional lots of nursery stock were found to be infected with the cankers, but the loss was never serious or alarming. The disease in most cases was evidently contracted during heeling in, judging from the fact that the cankers were scattered along the entire length of the tree and mostly on one side. There was little opportunity, however, to trace the matter to its source until the winter of 1921. This was a season of excessive rainfall in California, beginning early in November. During January and February there was a very heavy loss from this disease in nursery stock, particularly in the Sacramento Valley where it amounted to hundreds of thousands of dollars within a few weeks. Perhaps the greatest damage was in apricots and peaches, although almonds and plums, as well as apples and pears, were almost equally involved. It was evident at this time that much infection had occurred in the nursery row before the trees were dug, especially where they were small and thickly set, from splashing up of the surface soil. June-budded apricots and peaches were the worst infected, practically every tree in large blocks being cankered just above the bud. Four or five cankers to a tree were a common occurrence. The heaviest loss occurred in trees heeled in in exposed trenches. Isolations were readily made from such cankers while the bark was fresh; and by taking from the wood underlying the outer edge of the canker, cultures have often been secured after the bark was badly dried. Both strains A and B were found in such material, but the effect produced was the same in all cases.

INFECTION IN BEARING PRUNE AND PEACH TREES

During 1921 and 1922 many large trees in full bearing were killed or badly injured by large cankers at the crown, often girdling the tree. The condition was similar to that often attributed to winter killing or sun scald, and was no doubt largely contributed to by the unusual extremes of temperature and moisture occurring during this period. In making cultures from such cankers, however, pythiaceous strains were isolated from prune and peach orchard trees from three to eight years of age. These cases were as follows: Prune tree about five years old from Butte County, characteristic of a general condition in the orchards of this district in the winter of 1921. The tree was in good condition the year before, but the foliage withered

after starting to leaf out in the spring. More than fifty per cent of the trees in some orchards were in this condition. The canker, just above the crown, was mostly covered by heavy soil washed up by the rains. In the tree cultured the canker was five or eight inches wide and nearly girdled the tree. The bark was slightly sunken in the older parts of the canker, but aside from this and slight gumming the bark was fairly normal in appearance on the outside. On peeling away the outer bark, the affected area was seen to have much the same appearance as in Pythiacystis cankers on nursery trees, viz., a white, rather cheesy consistency in the central, older portion of the canker, the color turning darker with age, the border of the canker somewhat marked by zonate bands from light brown to translucent at the outer margin. The bark was affected down to the wood over most of the canker, which was active in spots at this time. Cultures were obtained in nutrient agar slants after twenty days. The fungus isolated, strain C, is discussed elsewhere. We have had better success in isolating the fungus from old, dried-out cankers by planting in agar slants than by any other method, as in many cases it pushes down and beyond the surface growth of other organisms.

In the latter part of December, 1920, a peach tree five or six years old showing crown canker of a similar nature was also cultured with positive results. This tree was obtained from Placerville and represented a common condition at the time in that region. The stem was about 2½ inches in diameter at the point of infection. The canker was mostly on one side and seemed to be of long standing. The lower portion was already invaded by various saprophytes, but the upper margin was still actively pushing out and showing the characteristic zonation. From this tissue cultures of the fungus were readily obtained, as described elsewhere under strain D.

Following this experience, an effort was made to determine the occurrence and seriousness of this infection in deciduous fruit trees of all ages.

INOCULATIONS IN BEARING TREES

On January 27, 1922, the following inoculations were made in Berkeley trees. 1. A cherry tree (*Prunus avium*) eight or ten years old, perfectly sound and growing rapidly, trunk 15 inches in circumference. 2. Apricot (*Prunus armeniaca*) eight or ten years old, a large tree, but with bark more or less sunburned. 3. Plum tree (*Prunus domestica*) 13 inches circumference. 4. Apricot, badly sunburned, 12 inches circumference. 5. Apricot tree 9 inches circumference, trunk in poor condition.

Tree 1 Inoculated with strain C, from bearing prune tree.
" 2 inoculated with strain A, from peach nursery tree.
" 3 inoculated with strain C.
" 4 inoculated with strain D, from bearing peach tree.
" 5 inoculated with strain D.

The inoculum was taken from potato agar cultures five days old, all showing abundant growth on the surface. The trunks were first sponged with formaldehyde, then with sterile water. Two or three stabs were then made in the bark just above the rootstock on the north side of the tree, using sterile scalpels pointed downward at an abrupt angle, the cut extending

FIG. 2. Lesion produced on sweet cherry tree by inoculation with strain C.

to the wood. Considerable mycelium was then inserted into each cut on the point of a sterile needle, tucking it well into the cut. Check cuts were made on each tree a little to the right and facing the north side, the inoculations to the left, with three or four inches between. The inoculations were not bound or covered in any way. Soil from another part of the yard

was piled rather high about the tree without coming into actual contact with the cuts. Two days later, rain and snow fell for about twelve hours. The rainfall for the period was rather moderate after the middle of February.

Results on May 24, 1922. On tree 2 the fungus had failed to grow and the cuts were practically healed. Trees 3, 4, and 5 showed small cankers. All were gumming considerably and showed the characteristic tissue discoloration. On tree No. 1, under closer observation on account of its better condition, the canker was found to be progressing more rapidly with profused gumming, as shown by figures 2 and 3. The fungus was recovered from the cankers in Nos. 1, 4, and 5 only. The checks showed no infection in any case. At the end of this period the cankers were cut out and the wounds treated.

FIG. 3. Outline of lesion produced on sweet cherry tree in four months by inoculation with strain C.

INOCULATIONS AT MOUNTAIN VIEW

On February 2, 1922, the following trees were inoculated by Mr. Bert Rudolph, at Mountain View, Santa Clara County, using the same strains: three mature apricot trees, one with each strain; two-year-old trees of plum and peach in orchard form. In this experiment the inoculum was inserted under flap cuts made just above the crown, then covered with a sterile linen pad. These trees were destroyed before final records were made. When observed on March 10, the two-year trees were gumming profusely with every indication of active infection. The inoculations on older trees were not successful.

WALNUT CROWN CANKER OR ROOT ROT

In the spring of 1923 several specimens of crown canker or root rot of northern California black walnut trees (*Juglans hindsii* Jepson) were cultured for Pythiacystis, and from one of these a culture was obtained in nutrient agar slant. This work was done in May and June, very late for active infection, and with old cankers nearly girdling the tree. By cutting down to the wood, however, the fungus was secured after the tissue had been nearly a month in culture. It appears from our experience with larger trees that while the organism may die out during the summer throughout a large part of the canker, it lives over at scattered points along the margin in the outer wood layer, where it seems to find some protection. In this way cultures have often been secured from nursery trees in a very dry condition. The walnut canker was of the same general type described for bearing peach and prune. A number of trees affected in this way have been seen in San Joaquin County, where this root rot or canker constitutes rather a serious disease.

Walnut Inoculation. Owing to the dry winter of 1923–24, no inoculations were tried until December, 1924. Two trees were then inoculated at the crown, as described for other strains. One of these trees was a black walnut seedling, the other a grafted tree of unknown variety. Small cankers about two inches in diameter have developed to date.

DISCUSSION OF THE MORPHOLOGY OF FIVE STRAINS WHICH HAVE BEEN TESTED BY INOCULATION

A. Original nursery stock strain. This differs in no important character from the fungus causing brown rot of lemon and described by the writers as *Pythiacystis citrophthora*. No oospores or chlamydospores have been developed. Growth on agar media is almost entirely submerged and the production of conidia usually very scanty. Abundant sporangia are developed in liquid culture as it dries down along the edge. Sporangia are

extremely variable as to size and shape. We may mention here an isolation from peach nursery stock showing typically blunted sporangia as in *Phytophthora syringae*, but no oospores.

B. From almond and other nursery stock infections. A strain similar to *P. citrophthora*, but producing abundant oogonia and antheridia in potato agar. The oospores average 30–40 μ, running up to 44 μ in diameter, thick-walled, with both paragynous and amphigenous antheridia.

FIG. 4. Oospores of strain B. Inset shows individual with amphigenous antheridium.

C. From crown canker of bearing prune tree. While oogonia are always present, sporangia are comparatively rare. In potato and nutrient agar the mycelium develops a heavy, white, aerial growth an inch or more above the medium and penetrates somewhat below the surface. The oogonia are scattered thickly through the lower portion of the aerial mycelium, which is straight, coarse, not profusely branched. The oogonia are small, rather thick-walled, developed on slender stalks. The average size is about 16–18 μ in potato agar. The antheridia are small, club-shaped, slender stalked, and may meet the oogonium at any point on its surface. The strain

develops occasional bodies slightly larger than the usual oospore, with thick walls showing distinct radial striations. These may be oospores, but their connection with an antheridium has never been observed. The sporangia from this strain, produced in one instance only by drying down liquid cultures, are lemon-shaped, averaging about 16 μ in length, with distinct papilla.

D. From crown canker of bearing peach. This strain has considerable resemblance to strain E from walnut in that abundant sporangia are produced on the surface of potato agar cultures after three or four days, the mycelium becoming distinctly tuberculate and growing only slightly above the medium. So far, however, it seems to differ from strain E in that oospores are scarce in potato agar, perhaps one being found in several mounts, widely scattered among the sporangia. The antheridia are both paragynous and distinctly amphigenous. Sporangia are almost spherical in most cases, with prominent papilla, this being the most striking characteristic of the strain. The average diameter of the sporangia is about 30–42 μ, although many are smaller. The average diameter of oospores is about 28–34 μ. This strain develops abundant chlamydospores on potato agar after three or four weeks.

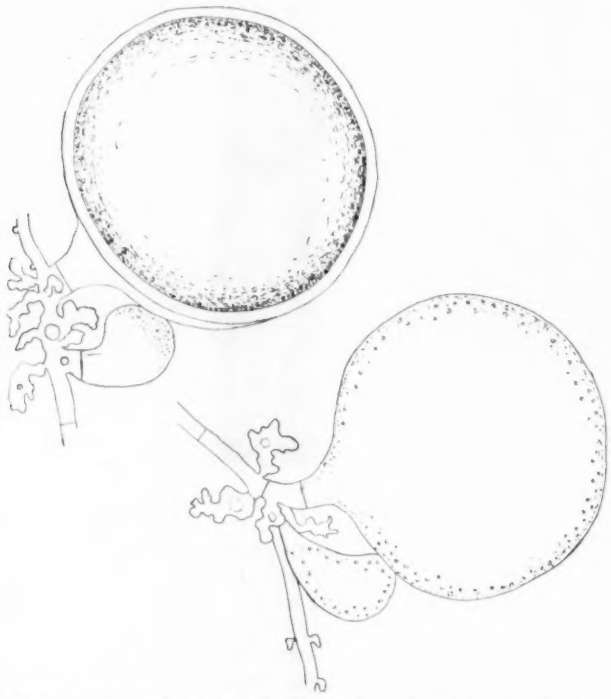


FIG. 5. Oogonia and antheridia of strain E from walnut.

E. From crown canker of black walnut. The antheridia of strain E are prominent and are mostly paragynous, although occasionally they appear to surround the oogonial stalk. The base of the antheridial stalk seems typically very near that of the oogonium, and in most cases the antheridium comes in contact with the oogonium just to one side of its base, as in figure 5. The latter characteristic is described by Rose (54) for his strawberry strains. On potato agar a scanty aerial mycelium, about 5 mm. above the medium, quickly develops tuberculate masses, as described by Rosenbaum (30) for *P. syringae*. The tuberculate mass usually surrounding the oogonium is figured in its simplest form and in cross section. The readiness with which fruiting bodies are produced is extremely variable, but usually when inoculum from an old culture is planted in fresh potato glucose agar, an abundance of both oogonia and sporangia is quickly formed on the surface (Fig. 6). The sporangia separate very readily from the stalk at the

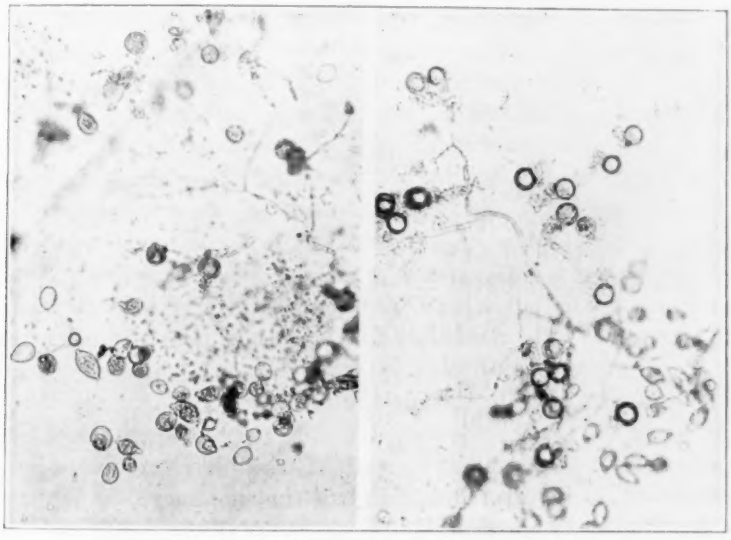


FIG. 6. Oospores and conidia of strain E from same culture.

cross wall just below the base, as in *P. infestans* and other species. In fact, it is often difficult to find the smaller ones still attached. The oospores average from 25 to 30 μ in diameter. The sporangia are exceedingly variable in size and shape. Of the larger sizes, about 25 x 46 μ is most common, tapering from the base; although a more slender lemon shape, about 27 x 43 μ, is abundant. Of the small sizes, which are more numerous, a broad oval to nearly circular is a common type, the average length about 25 μ. Many of these have the papilla on one side.

Both the peach and walnut strains (D and E) seem to be typical of *Phytophthora cactorum*, although strain D has produced very few oogonia. The prune strain (C) is of the cactorum group according to Rosenbaum, but has the general character of the genus *Pythium*, as now understood.

Under strain A, from nursery stock (considered as typical *Pythiacystis citrophthora*), have been placed all forms developing sporangia only and these sparsely on potato agar but rather abundantly at the edge of liquid media. Some of the isolations show the lemon-shaped conidia typical of *P. citrophthora*, others distinctly pear-shaped as in *Phytophthora syringae*. The mycelium in the host plant is entirely sterile.

TAXONOMY

The comparative study of these and other strains of this group over a period of several years, together with consideration of the literature, creates a strong doubt as to the validity of the present distinctions between the genera *Pythium*, *Phytophthora*, and *Pythiacystis*. Fitzpatrick (49) has proposed merging *Phytophthora* with *Pythium* on account of the occurrence of species of the latter in which both the characteristic types of zoospore formation occur—*i.e.*, differentiation within the sporangium and that outside in a vesicle. The latter is the only definite morphological character on which *Pythium* is based. Species of *Phytophthora* are also known in which a thin-walled vesicle is formed at the mouth of the sporangium (Rosenbaum, 30). The evanescent, colorless texture of the sac renders observation of this in both genera difficult and inadequate. It is particularly evident in our walnut strain that such a membrane exists. Sporangia of a slow-growing culture, as on a glass slide in a moist chamber, are good for such study. Here, after several days in scant moisture, sporangia are often found, the contents of which had begun to emerge, then receded back into the sporangium. In such cases a distinct sac may be clearly seen at various initial stages extending out as a stretched lining from the ends of the sporangium wall to the tip. It is also true that the matter of habitat, once considered of some importance in distinguishing *Pythium* from *Phytophthora*, is now valueless, as so many soil organisms have been placed in the latter genus.

We have already discussed reasons for combining *Pythiacystis* with *Phytophthora*, based on the abundance of soil-inhabiting species and strains of the latter which are now known. We have, therefore, at present a series of species and strains intermediate between all three of these genera and between which no sharp distinction can be drawn upon the basis of the features which formerly were considered characteristic. It seems to us that the time is not yet ripe for deciding whether *Pythiacystis* should be merged with *Pythium* or with *Phytophthora*, or whether all three should be merged

together, or an entirely new sub-grouping be established. This is on account of the fact that there is so much variation in size, form, type, and occurrence of reproductive bodies and that so many intermediate forms are continually being discovered. A restricted genus *Phytophthora* based on the original type, *P. infestans* de Bary, with quite definite conidiophores and typical aerial, parasitic habitat would be fairly logical at present. This, of course, comes back to the original distinction between *Phytophthora* and *Pythiacystis*. As suggested by Fitzpatrick (49), the name *Pythium* has precedence over *Phytophthora* and should be retained in event of combining the genera under consideration.

SUMMARY

1. Since the genus *Pythiacystis* was described by the writers in 1906, several new species of *Phytophthora* have been described, representing types which obscure the distinction between these genera.

2. These species are characterized by the occurrence of a variety of strains and morphological variations which make specific and sub-generic delimitation somewhat uncertain.

3. A number of pathogenic strains have been found in northern California which form a closely connecting series between typical *Pythiacystis citrophthora* and fungi of the *Phytophthora cactorum* type.

4. A brief description of five of these strains is given. Four of them resemble *Phytophthora cactorum*, with paragynous antheridia, and in three of these the amphigenous type is also found. The fifth strain is similar to typical *Pythiacystis citrophthora*, having conidia only. None of these strains is selective as to host, but all seem to produce about the same symptoms.

5. Fungi of this type cause crown or trunk canker in nursery and orchard trees of pear, peach, almond, apricot, cherry, plum, prune, and black walnut. A great deal of damage is done to nursery trees by diseases of this sort, particularly in wet seasons.

6. In regard to taxonomy, various forms described by the writers and others appear to obscure the present distinction between the genera *Pythium* Pringsheim, *Phytophthora* de Bary, and *Phythiacystis* Smith and Smith. If these genera are merged, the name *Pythium* would have precedence.

7. The changing of *Pythiacystis* to either *Phytophthora* or *Pythium* is not proposed at present owing to the doubtful status of the delimitation of these genera.

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LITERATURE CITED

1. LEBERT, H., and F. COHN. Ueber die Fäule der Cactusstämme. Beiträge zur Biologie der Pflanzen **1**: 51–57. 1870.
2. HARTIG, ROBERT. Die Buchencotyledonen-krankheit. Ztschr. Forst. u. Jagdw. **8**: 117–123. 1876.
3. BARY, ANTON DE. Zur Kenntniss der Peronosporen. Bot. Ztg. **39**: 521–530, 537–544, 553–563, 569–578, 585–595, 601–609, 617–625. 1881.
4. BREDA DE HAAN, J. VAN. De bibitziekte in de Deli-tabak veroorzaakt door *Phytophthora nicotianae*. Meded. Lands. Plantentuin. **15**: 107 p. 1896.
5. KLEBAHN, H. Eine neue Pilzkrankheit der Syringen. Cent. Bakt. II, **15**: 335–336. 1905.
6. OSTERWALDER, A. Die Phytophthorafäule beim Kernobst. Cent. Bakt. II, **15**: 435–440. 1906.
7. SMITH, RALPH E., and ELIZABETH H. SMITH. A new fungus of economic importance. Bot. Gaz. **42**: 215–221. 1906.
8. ———— The brown rot of the lemon. Cal. Agr. Exp. Sta. Bul. 190: 1–72. 1907.
9. KLEBAHN, H. Krankheiten des Flieders. p. 75. 1909.
10. MARCHAL, E. Sur une maladie nouvelle du poirier. Bul. Soc. Roy. Bot. Belgique **45**: 343–344. 1909.
11. MAUBLANC, A. Les maladies des plantes cultivées dans les pays chauds. Maladies du cacaoyer. Agr. Prat. Pays Chauds **9**: 314–324, 393–407, 472–479. 1909.
12. BUBAK, F. Die Phytophthorafäule der Birnen in Böhmen. Ztschr. Pflanzenkrank. **20**: 256–261. 1910.
13. COLEMAN, L. C. Diseases of the Areca palm (*Areca catechu* L.) I. Koleroga or rot disease. Ann. Myc. **8**: 591–626. 1910.
14. OSTERWALDER, A. Die Phytophthora-fäule bei Erdbeeren. Landwirt. Jahrb. Schweiz. **19**: 320–321. 1912.
15. ———— Das Absterben von Veredlungen, Verursacht durch Phytophthora. Landw. Jahrb. Schweiz. **19**: 321–322. 1912.
16. DASTUR, JEHANGIR FARDUNJI. On *Phytophthora parasitica* n. sp. Mem. Dept. Agr. India Bot. Ser. **5**: 177–231. 1913.
17. FAWCETT, H. S. Two fungi as causal agents in gummosis of lemon trees in California. Phytopath. **3**: 194–195. 1913.
18. ———— Two fungi as causal agents in gummosis of lemon trees in California. Monthly Bul. Cal. State Comm. Hort. **2**: 601–617. 1913.
19. PETHYBRIDGE, G. H. On the rotting of potato tubers by a new species of *Phytophthora* having a method of sexual reproduction hitherto undescribed. Sci. Proc. Royal Dublin Soc., n. s., **3**: 529–565. 1913.
20. SCHOEVERS, T. A. C. Eene voor Nederland nieume Seringenziekte, veroorzaakt door *Phytophthora Syringae* Klebahn. Tijd. Plantenz. **19**: 41–64. 1913.
21. ROSENBAUM, J. *Phytophthora arecae* (Colem.) Pethyb. causing rot of potato tubers. Phytopath. **4**: 387. 1914.
22. WILSON, G. W. Studies in North American Peronosporales–V. A review of the genus *Phytophthora*. Mycologia **6**: 54–83. 1914.
23. FAWCETT, H. S. The known distribution of *Pythiacystis citrophthora* and its probable relation to Mal di Gomma of citrus. Phytopath. **5**: 66–67. 1915.
24. ROSENBAUM, J. Phytophthora disease of ginseng. N. Y. (Cornell) Agr. Exp. Sta. Bul. 363. 65–106. 1915.

25. SAWADA, K. Two new diseases of Welch onion and egg plant due to fungi belonging to the genus *Phytophthora* (*P. allii*, *P. melongenae*). Sp. Rept. Agr. Exp. Sta. Formosa **11**: 1–190. 1915. (In Japanese, see 32.)

26. SMITH, ELIZABETH H. Pythiacystis infection of deciduous nursery stock. Phytopath. **5**: 317–322. 1915.

27. FAWCETT, H. S. A Pythiacystis on avocado trees. Phytopath. **6**: 433–435. 1916.

28. WHETZEL, H. H., and J. ROSENBAUM. The Phytophthora rot of apples. Phytopath. **6**: 89–90. 1916.

29. BARRETT, J. T. Pythiacystis related to Phytophthora. Phytopath. **7**: 150–151. 1917.

30. ROSENBAUM, J. Studies of the genus *Phytophthora*. Jour. Agr. Res. **8**: 233–276. 1917.

31. SHERBAKOFF, C. D. Buckeye rot of tomato fruit. Phytopath **7**: 119–129. 1917.

32. TANAKA, T. New Japanese fungi. Notes and translations II. Mycologia **9**: 249–253. 1917. (Translation of 25.)

33. DEMANDT, E. Untersuchungen über Kanker und Braunfäule am zamoanischen Kakao. Ztschr. Pflanzenkrank. **28**: 241. 1918.

34. MURPHY, P. A. The morphology and cytology of the sexual organs of *Phytophthora erythroseptica* Pethy. Ann. Bot. **32**: 115–153. 1918.

35. STEVENS, H. E. Citrus diseases. Fla. Agr. Exp. Sta. Rept. (1916) **17**: 10R–11R. 1918.

36. Anonymous. A disease of pears new to the continent of America. *Phytophthora cactorum*. Agr. Gaz. Canada **6**: 951–952. 1919.

37. PETHYBRIDGE, G. D., and H. A. LAFFERTY. A disease of tomato and other plants caused by a new species of *Phytophthora*. Sci. Proc. Roy. Dublin Soc. n. s **15**: 487–505. 1919.

38. WORWALD, H. A Phytophthora rot of pears and apples. Ann. Appl. Biol. **6**: 89–100. 1919.

39. BEWLEY, W. F. "Damping off" and "foot rot" of tomato seedlings. Ann. Appl. Biol. **7**: 156. 1920.

40. CLINTON, G. P. Injuries and diseases of plants. Conn. Agr. Exp. Sta. Bul. 222: 397–482. 1920.

41. FAWCETT, H. S. *Pythiacystis* and *Phytophthora*. Phytopath. **10**: 397–399. 1920.

42. REDDICK, D. A fourth Phytophthora disease of tomatoes. Phytopath. **10**: 528–534. 1920.

43. ROSENBAUM, J. Infection experiments on tomatoes with *Phytophthora terrestria* Sherb. and a hot water treatment of the fruit. Phytopath. **10**: 101–105. 1920.

44. BEACH, W. S. A Phytophthora crown rot of rhubarb. Phytopath. **11**: 55–56. 1921.

45. ———— The crown rot of rhubarb caused by *Phytophthora cactorum*. Pa. Agr. Exp. Sta. Bul. 174. 1–28. 1922.

46. BRUYN, H. L. G. DE. The saphrophytic life of *Phytophthora* in the soil. Meded. Landbouwhoogesese. Wageningen **244**: 1–37. 1922.

47. LAFFERTY, H. A., and G. D. PETHYBRIDGE. On a Phytophthora parasitic on apples, which has both amphigynous and paragynous antheridia; and on a large species which shows the same phenomenon. Sci. Proc. Roy. Dublin Soc. n. s. **17**: 29–43. 1922.

48. FAWCETT, H. S. Gum disease of citrus trees in California. Cal. Agr. Exp. Sta. Bul. 360: 370–423. 1923.

49. FITZPATRICK, H. M. Generic concepts in the Pythiaceae and Blastocladiaceae. Mycol. **15**: 166–173. 1923.
50. GODFREY, GEO. H. A Phytophthora foot rot of rhubarb. Jour. Agr. Res. **23**: 1–26. 1923.
51. HOTSON, J. W., and LENA HARTGE. A disease of tomatoes caused by *Phytophthora mexicana* sp. nov. Phytopath. **13**: 520–530. 1923.
52. REINKING, O. A. Comparative study of *Phytophthora faberi* on coconut and cacao in the Philippine Islands. Jour. Agr. Res. **25**: 267–284. 1923.
53. BRUYN, HELENA L. G. DE. The Phytophthora disease of lilac. Phytopath. **14**: 503–517. 1924.
54. DUFRENOY, J. Les maladies des arbres causées par des champignons du type Phytophthora. Rev. Bot. Appl. **4**: 803–809. 1924.
55. ROSE, DEAN H. Leather rot of strawberries. Jour. Agr. Res. **28**: 357–375. 1925.
56. TUCKER, C. M. Coconut bud rot experiments in Porto Rico. Science n. s. **61**: 186–187. 1925.

SPRAY INJURY TO APPLE

H. C. Young and R. C. Walton

With Plate XIV

The data here presented were secured in connection with an investigation of the fungicidal properties of sulphur. This investigation was conducted under the direction of the Crop Protection Institute in administering a research fund provided jointly by the Freeport Sulphur Company, the Texas Gulf Sulphur Company, and the Union Sulphur Company.

The studies here recorded were made possible through the generous cooperation of the New York, Geneva, Agricultural Experiment Station and especially through the personal assistance of Dr. R. W. Thatcher, Director, and Professor P. J. Parrott, Chief in Entomology.

The injury to foliage and fruit resulting from spraying during the season of 1923 was exceptionally serious in many localities. Severe cases of such injury have occurred occasionally since spraying began. This has been true when Bordeaux mixture and lime-sulphur were used, but rarely have the wettable and "dry mix" sulphurs and sulphur dusts caused injury as severe as during the past season. This was especially true in the states of New York and Pennsylvania where, in general, the weather conditions were not extreme, except for one week of very hot weather in June. On the whole, the season from June to August, inclusive, was quite dry. During late July injury was observed following the use of many kinds of spray materials. At this time an effort was made to determine the types of spray injury to fruit and leaves and the underlying cause or causes of such injury.

Several attempts have been made to determine the cause of spray injury, but because of the complex chemical changes that take place in spray mixtures both before and after application and the various climatic conditions that affect these changes, little definite progress has been made. The physiological condition of the tree seems also to be an important factor.

One of the first comprehensive studies of spray injury was made by Wallace (9). He diagnosed several types of injury the most common of which was tip burning. This, he states, results from the accumulation of the spray liquid at the tip. Other types noted were: (1) injury following fungus infection; (2) the scorching of large parts of the leaf surface, which results when foliage is drenched; (3) Bordeaux injury, which may appear at first as very small spots gradually increasing in size. It is the opinion of the author cited that the action of lime-sulphur and Bordeaux are different, the action of the former being immediate, that is, within three

or four days, while that of the Bordeaux is more moderate at first but extends over a much longer period of time. The author concludes that there is no correlation between the weather at the time of, or after application and the resulting injury; that possibly the weather conditions previous to the time of application may so influence the foliage as to make it more or less susceptible to lime-sulphur spray injury; that the agent active in causing lime-sulphur injury is doubtless the soluble sulphur in the form of a calcium sulphide.

Safro (5) is of the opinion that the concentration of the soluble sulphides of lime-sulphur is the important factor in burning. He states that the only ingredients in lime-sulphur that are instrumental in burning are the polysulphides and thiosulphate. He states that the specific gravity of any lime-sulphur solution is not an index to the amount of burning that can be expected. "Though the amount of soluble sulphides in the spray may be known, other factors still remain to modify, sometimes radically, the power of a spray to produce injury to foliage or fruit."

In 1912, Stewart (7) studied sulphur-arsenical spray injury. He states that the chief factors concerned in this type of spray injury are: (1) the character of the arsenical, (2) the purity of the sulphur solution, (3) the soundness of the epidermis on foliage and fruit, *i.e.*, its freedom from punctures and breaks due to insects or fungous attacks, (4) density and abundance of the application, (5) the size of the drops that collect, and the time required for their evaporation, (6) differing resistance of varieties, (7) excessive temperatures, (8) general weather conditions. The best method that he found for eliminating injury was by using the ortho-arsenate of lead which is the tri-plumbic arsenate, Pb_3 ($As O_4$). The pyro, $Pb_2 As_2 O_7$, and acid arsenate, Pb H $As O_4$, are stable only in acid mixtures while the ortho is more stable under neutral and alkaline conditions. The addition of sugar of lead frequently reduces burning in arsenical lime-sulphur mixtures.

Patten and O'Meara (4) studied injuries following the use of calcium and magnesium arsenates.

Frequently burning of foliage can be traced directly to the arsenicals. Fernald and Bourn (2) studied arsenical burning and concluded that neutral lead arsenate was the safest material, especially in clear weather; that clear weather spraying is safer than the cloudy weather; that spraying can be done safely at high temperatures if the humidity is low; that spraying can be done when the humidity is high if the temperature is low. Krout (3) also attributes much to the weather and states that apples should never be sprayed when the temperature and humidity are both high, as burning of foliage is almost certain to result. He states further that sulphur dusts have never burned the foliage, while burning with copper-lime dust is frequent.

Investigations extending through a period of 10 years and involving an application of various arsenical mixtures to 10,000 separate plants have been reported by Swingle, Morris and Burke (8). They attributed most of the injury resulting from spray mixtures containing arsenicals to the soluble arsenic. They have found it quite difficult to make exact statements concerning the toxicity of the different arsenicals because of the variability of their composition. One might theoretically expect ortho, meta and pyro arsenates, each in the form of monoplumbic, diplumbic and triplumbic salts, or in other words, a total of 9 possibilities. While this is not actually possible yet commercial lead arsenates are not single salts of lead and arsenic acid but a mixture of different lead arsenates. A company's product may change within a short period, even that prepared at the same factory. These authors conclude that arsenic trioxid, which was formerly thought to burn in all concentrations, is not so dangerous, provided it is applied promptly after mixing with water. Leaves injured by arsenicals show evidence of this by turning brown. They state that the injury to foliage is practically through the lower epidermis, regardless of the number of stomata in the two surfaces. This injury is influenced more by humidity than by temperature.

In this short review of work on spray injury no very definite conclusion can be obtained. The indications are that much of the injury resulting from lime sulphur-lead arsenate mixtures is due to the soluble arsenicals, but not all may be. Lime-sulphur alone will burn foliage. Bordeaux mixture alone will cause severe burning. It is to be admitted that climatic factors play an important rôle. In determining the underlying cause of injury not only the above factors must be considered, but in addition, the chemical changes taking place before and after application of any mixture must be considered. The question to be answered is why, under certain conditions, do many sprays burn foliage and russet fruit.

The work reported in this paper was begun in the latter part of July, 1923, when injury to fruit and foliage was at its height in New York and Pennsylvania. It was realized that many points could not be determined because of the lateness of the season. The report contained herein gives a summary of the types of injury induced by the various spray mixtures and the results of some experiments on the chemical compounds thought to be responsible for injury. The experiments were conducted in several orchards in the vicinity of Geneva, New York, and in Adams County, Pennsylvania.

TYPES OF INJURY

I. *Edge burn* was the most common type of injury found. It is characterized by injury to the edge of the leaf, which parts turn brown and may finally slough off. Various degrees of injury result, from a small

area to the entire leaf edge. Frequently only a small portion of the leaf surface remains on each side of the mid rib. This type of injury does not appear to be correlated with high temperatures. It is as severe on one side of the tree as the other, that is, relative to north, south, east or west. It appears to be more severe in the paths of the spray gun. It occurred alike on all the varieties tested, namely, Greening, Rome Beauty, Winesap, Stayman Winesap, York Imperial, Northern Spy, Baldwin, Black Twig, King, Wealthy and Dutchess. The injury appears within three or four days after application of spray. It is not confined to the use of lime-sulphur, but occurs in somewhat less degree and in modified form with colloidal sulphur and slightly with precipitated sulphur. With these sprays and with dry mix the injury largely takes the form of a moderate tip burn. This type is shown in plate XIV, D, and a record of occurrence is given in table 1.

II. *Scald* is a type of spray injury found on both foliage and fruit following the use of various sulphur sprays applied in extremely hot weather. Scald on the foliage differs from edge burn in that usually larger areas of the leaf are involved and very frequently the entire leaf. The areas are, as a rule, lighter brown than the edge burn injuries and are not in the form of small spots, as is the case with Bordeaux injury, but are large. They may extend to the margin of the leaf or may involve the entire surface.

On fruit the scalded areas are light brown in color with irregular margins. They may be largely superficial and may callous over and be scarcely noticeable at picking time. Seriously injured areas have a more definite margin, are darker in color and may involve the skin and part of the flesh beneath. The injury on Stayman Winesap appeared quite different from that on Rome Beauty.

Both the foliage and the fruit types of scald are found much more abundantly on the lower half of the trees. Whether this is due to greater force of application from the spray nozzles or to a greater amount of spray material on the fruit and foliage is not known. Scald appears to be correlated with extremely high temperatures. It is always found on the southern exposures of the trees and never on the northern, unless an apple or leaf is so located as to receive the direct rays of the sun. Various sulphur sprays will produce it, but in the cases observed in 1923 it was much more serious on trees sprayed with commercial lime-sulphur. Dry mix, colloidal and precipitated sulphurs produced it, but only to a slight extent. 90–10 dust produced it and pure sulphur dust caused it during the season of 1923, if the trees were dusted when the temperature was around 100° F. in the shade. There does not seem to be any considerable difference in susceptibility among varieties. It appears probable that scald

is a sulphur burn and its severity is correlated with temperature conditions. Bordeaux mixture did not cause it in 1923, whereas lime-sulphur applied the same day in the same orchard produced serious injury. Arsenate of lead was used in both materials. Scalded leaves and fruit are shown in plate XIV, A, B, and C, and the relative occurrence is indicated in table 1.

III. *Yellow leaf* is a further type of spray injury. It appears within a week or ten days after lime-sulphur has been applied in late July and August. This type of spray injury is characterized by the leaves turning yellow or partially yellow and falling off. A greenish and yellowish mottling is characteristic. In some orchards the majority of the yellow leaves are the smaller, older ones which appear early in the spring around the blossom clusters. In other orchards the reverse has been noted, the yellow leaves being mainly the later, larger ones. One fact stood out prominently in 1923 in Adams County, Pennsylvania, namely, that the tip leaves were able to withstand this type of spray injury. Seldom were the tip leaves affected. Varieties differ in their susceptibility to this type of injury. In New York State the variety most severely affected when lime-sulphur was used was the King. In this case the early August spray caused 60 per cent of the leaves to fall off. Greening, Northern Spy and Baldwin varieties were less severely injured, approximately 15 per cent of their leaves falling after this spray. No yellow leaves appeared on the Rome Beauty when lime-sulphur was used. The same varietal susceptibility was noted in Adams County, Pa. The York Imperial was seriously affected, also Grimes Golden and Delicious, while the Stayman Winesap and Black Twig showed very little yellow leaf following any of the sulphur sprays used or following Bordeaux. In some cases varieties resisted lime-sulphur but did not the colloidal or precipitated sulphurs. This was the case with Rome Beauty in New York State.

Yellow leaf is, in the main, a mid-summer spray injury. It has been pronounced in southern Pennsylvania during the summers of 1922 and 1923, both of which were unusually dry. The same was true for New York conditions. It is not known whether the drought was a contributing factor, but it seems possible that results noted would be more likely to occur in dry weather than in seasons of normal precipitation. More definite observations are contemplated in an attempt to correlate weather conditions with this type of injury. Yellow leaf is frequently attributed to frost injury. This is not thought to be the case, although frost injured leaves are frequently the first to become affected. Leaves injured by frost are always puckered and when a leaf in this condition is bent the epidermis breaks so that the bright green tissues beneath are exposed. Yellow leaf is shown in plate XIV, E, and the prevalence of this type of injury with various spray materials is set forth in table 1.

IV. *Goose neck.* Goose neck is a term which was given by Dr. Waite, of the Bureau of Plant Industry, U. S. D. A., to a type of injury. This type is characterized by a longitudinal arching of the leaf, sometimes barely noticeable and at other times so pronounced as to produce a rolling.

Leaves but slightly arched frequently show no necrotic areas or other apparent injury, or at most, only a very slight amount. Severe arching is very frequently accompanied by more or less serious edge burn indicating that possibly the edge burn type of spray injury can be responsible for goose neck. However, this is certainly not always true because cases have been noted in New York and Pennsylvania where severe arching occurred on check trees. These trees were severely infested with the Red Bug and the scars produced by this insect were especially numerous on the goose neck leaves. Arched leaves frequently show slight injury to the mid-rib and it is thought that different kinds of injury may be responsible for this type.

Various types of goose neck are shown in Fig. 1, and the amount of this type of injury is indicated in table 1.

V. *Injury Following Apple Scab.* This is a type which has been recognized for years but about which little seems to be known and little has been written. It is characterized by a solid circle of light brown, necrotic leaf tissue in the center of which is the dark scab spot. The entire area looks not unlike a large frog-eye spot after secondary growth has taken place, with the exception, however, that the light center, so characteristic of the frog-eye spot, is lacking and in its place is the larger and darker scab area (Plate XIV, F).

The writers have no authentic data as to the cause of such injury. In 1923, it was quite abundant where certain sprays were used. For instance, in plots which received barium sulphide and arsenate of lead, both in New York and Pennsylvania, such injury was abundant. In another Pennsylvania orchard of Stayman Winesap it was very plentiful in a plot which received arsenate of lead only, up to and including the 10-day spray. In an adjoining plot which received lime-sulphur only, up to and including the 10-day spray, there was practically no injury of this type. Both plots were treated alike after the 10-day spray, receiving one application of sulphur dust. In this orchard the indications are that the arsenate of lead is responsible but this cannot be definitely stated until further work has been done.

VI. *Leaf roll* is thought by some to be a type of spray injury. This is characterized by the margins of the leaf rolling upward. The authors could collect no data which would lead them to believe that it was a result of spraying.

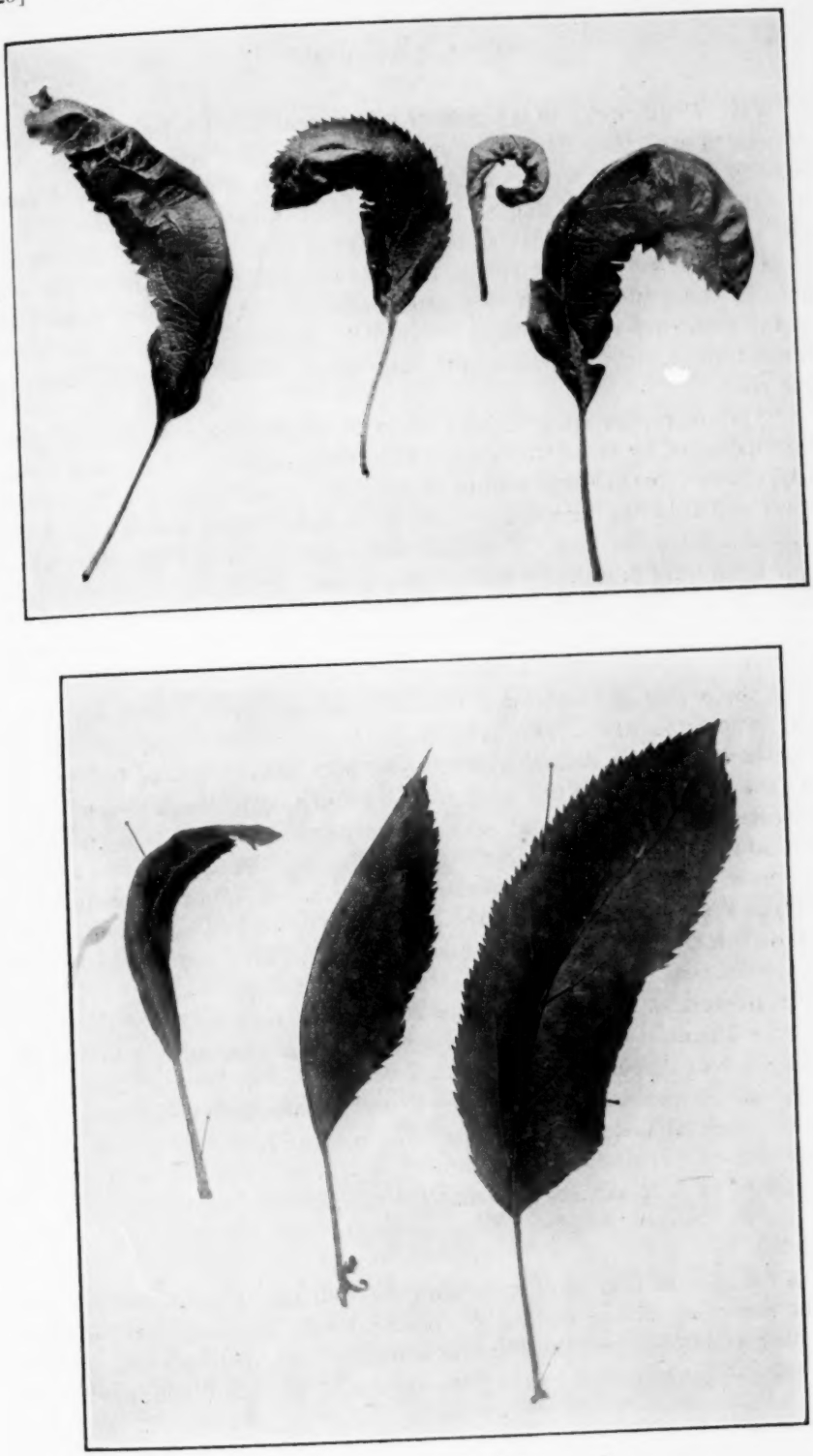

FIG. 1. Various types of goose neck.

VII. *Fruit russeting* is a type of injury generally known. It is usually associated with Bordeaux mixture or other copper sprays. During the summer of 1923 the writers became convinced that lime-sulphur can cause it. In Adams County, Pennsylvania, plots were sprayed with lime-sulphur and Bordeaux mixture, leaving one unsprayed plot as a check. The copper plots showed 50 per cent russet, the lime-sulphur plots 15 to 31 per cent and the check plots only a very small amount. In New York State plots of the Greening varieties were sprayed with lime-sulphur and Bordeaux. Lime-sulphur plots showed 10 per cent russet while Bordeaux showed 65 per cent.

Types of copper spray injury, other than those mentioned above, have been discussed by other workers. The authors observed that a long period may elapse after the application of a copper spray before the injury appears and that the injury may finally be much more severe than when sulphur sprays are used. One case was noted in New York State where pear trees were practically leafless two months after a mixture of copper and lime had been applied.

CAUSE OF SPRAY INJURY

In the review of literature it will be noted that various factors contributed to spray injury. There is no doubt in the minds of the authors that climatic factors are definitely correlated with some types of injury. In part the type of injury produced will be influenced by weather conditions. However, the weather alone will rarely cause burning. This must be induced by some ingredient in the spray mixture. This ingredient may be any one of many. It is not necessarily an arsenical. Most sprays that can be classed as fungicides may burn foliage under certain conditions. This was demonstrated during the season of 1923. If a spray has enough soluble material in it to kill fungi, it may burn foliage.

To prove that it is the soluble material in a fungicide that should be viewed with suspicion, a series of experiments were conducted with sulphur and certain of its compounds.

The first experiment was made by spraying commercial lime-sulphur on young peach foliage. Peach was selected because it is very susceptible to spray injury. Lime-sulphur, diluted 1 to 50, was applied with a hand sprayer at 11 A. M. on August 3rd. The temperature was about 88° F. in the shade. Severe foliage injury appeared within 36 hours, as was expected.

In contrast to this, trees were sprayed with precipitated sulphur prepared according to the method of Young (10). The sulphur was completely precipitated and the mixture contained only sulphur and calcium sulphate. This was used in the proportion of 5 pounds of sulphur to 100

gallons of spray mixture. This was the concentration used for apple spraying. Only slight injury to the foliage could be detected.

Another trial of the precipitated sulphur was made when conditions were thought to be more favorable for burning. At 1 o'clock on August 6th. two peach trees were heavily sprayed. The humidity and temperature were both high. That afternoon, about 4:30, it began to rain and continued to rain for an hour or more. The following day the sun was bright and temperature quite high. Only slight injury followed.

A number of experiments were conducted with colloidal sulphur prepared in various ways. The initial ingredients were sodium thiosulphate and sulphuric acid. When these are mixed colloidal sulphur is produced, also sodium sulphate and sodium thiosulphate. In one test the colloidal sulphur was centrifuged out and washed four times with distilled water. A mixture of this was sprayed on beans, potatoes, rose and tomato leaves. No burning resulted. Similarly colloidal sulphur washed one, two and three times was used. Burning on roses, tomatoes and beans was greatest with the material washed the fewest times. Separate experiments were made using sodium sulphate and thiosulphate with the result that each burned in concentration that was not toxic to fungi.

In the course of summer field work, when the colloidal sulphur was prepared on a large scale, it was impossible, because of the limitations of apparatus available, to free it of these soluble materials. There resulted frequent injury from its use on apple, and this injury was most severe on peach leaves.

DISCUSSION AND RESULTS

In our work evidence points to the soluble material in spray mixtures as being directly responsible for the burning of leaves and russeting of fruit. A spray mixture may contain one to many soluble compounds. Some of these soluble compounds are electrolytes of strong coagulating power. When such compounds come in contact with the protoplasms of the very active leaf cells injury may result, probably through plasmolysis and coagulation. This is particularly evidenced when the leaf is previously injured by fungi, thereby permitting the soluble fungicide to diffuse through the dead tissue to the living tissue.

When sprays are applied with a gun so that the mixture strikes the leaves with considerable force injury may result. Possibly the lower epidermis is weakened or injured and the cuticle partially penetrated by such force. This is not a new observation but has been noted ever since spraying began. If the spraying is not carefully done it often happens that one can tell where the spray nozzle or gun was located during spraying because of the paths of injury.

TABLE 1.—*Types of spray injury at Geneva, N. Y., caused by various spray materials; results expressed as percentages*

Kind of tree	Type of spray	Free from injury	Duplication	Edge burn			Goose neck with injury		Goose neck without injury		Scalding	Injury following scab	Yellow leaf	Total No. of leaves
				slight	mod.	sev.	slight	sev.	slight	sev.				
						Geneva Experiment Station Orchard								
Rome Beauty	Lime-sulphur	14.1	9.4	31.2	28.5	19.1		12.6			3.3	0.1	0.3	1,000
	Lime-sulphur and casein	14.0	16.4	38.2	29.4	17.4	3.6	8.4	0.4		7.7	0.0	0.9	1,000
	Sulphur glue spray 8–4–50	94.2	0.6	1.8	1.8	0.0	0.6	0.0	0.6	0.8	0.8	0.0	0.0	500
	Sulphur lead arsenate dust 90–10	93.6	0.0	2.4	1.4	0.2	0.8	0.2	0.8	0.0	0.8	1.4	0.0	500
	Precipitated sulphur	87.2	1.8	6.6	1.8	0.4	0.8	0.6	0.0	0.0	2.8	1.2	0.4	500
	Colloidal sulphur	41.6	16.9	28.5	15.9	9.9	3.8				1.9	0.37	14.6	1,100
						Wilson Orchard								
Greening	Lime-sulphur	35.3	19.5	47.1	6.3	1.2	0.4	1.1	1 8	0.1	0.7	0.0	15.4	1,000
	Lime-sulphur and casein, 1 lb. to 100	38.0	21.0	47.3	4.7	0.8	1.2	0.7	2.2	0.6	3.3	0.0	16.2	1,000
	Lime-sulphur and casein, 2 lbs. to 100	31.0	20.8	50.0	9.1	3.8	0.6	1.0	2.4	0.2	2.8	0.0	17.4	1,000
	Lime-sulphur and 1–40 glue	28.3	30.2	52.3	5.3	1.7	1.7	1.0	4.1	1.1	5.1	0.0	22.0	1,000
	Sublimed sulphur, No. 8, and casein spreader	88.0	0.0	0 0	0.0	0.0	0.0	0.0	2.8	0.4	0.4	0.0	0.0	500
	Same and 16 lbs. lime	82.2	0.2	0 0	0.0	0.0	0.0	0.0	5.2	0.2	0.0	0.0	0.0	500
	Precipitated sulphur	59.6	11.9	29.5	0.6	0.3	0.5	0.1	0.9		0.1	0.0	14.3	1,000
	Colloidal sulphur	47.0	11.4	41.8	5.2	2.0	0.5	0.0	0.1	0.1	0.1	0.0	12.5	1,000
	90–10 dust, regular schedule	81.4	0.2	0.0	0.0	0.0	0.0	0.0	4.2	0.2	0.0	0.0	0.0	500
	Check	84.2	0.0	0.0	0.6	0.0	0.0	0.0	2.4	0.0	0.0	0.0	0.0	500
						Post Orchard								
N. Spy	Lime-sulphur	41.8	12.9	44.1	6.1	1.1	1.1	0.7	0.0	0.0	1.5	0.0	16.4	1,000

Climatic factors influence the chemical activity of spray mixtures. Spray materials containing some form of sulphur which must be acted upon by climatic factors before it becomes fungicidal, rarely burns severely when used alone. Sulphur is believed to be toxic because of its oxidation product and this product is formed but slowly at ordinary temperatures. It is possible that at extremely high temperatures sulphur may burn, but such cases are rare. The effect of temperature on the chemical action of soluble materials is necessarily an important one. Undoubtedly the action is accelerated both in a chemical and physical manner.

Climatic factors undoubtedly can influence the burning action of lime-sulphur. This solution is extremely alkaline and remains so until the mixture is dry or acted upon by the CO_2 of the air. The complete change of lime-sulphur to precipitated sulphur and calcium sulphate requires from one-half to two hours. If this process is retarded and if conditions are favorable, the soluble sulphides may burn.

It is to be expected that the various soluble chemical substances contained in spray mixtures would cause different types of injury. It is the opinion of the authors that a sufficiently definite study of spray injury can be achieved only through correlating climatic records with a study of the soluble compounds contained in spray mixtures.

LITERATURE CITED

1. CLINTON, G. P. Spray injuries. [Bordeaux and lime-sulphur injury.] Rept. Connecticut Agric. Exp. Sta. **1911**: 360–363. 1912.
2. FERNALD, H. T., and A. I. BOURNE. Injury to foliage by arsenical sprays. Massachusetts Agric. Exp. Sta. Bull. 210: 89–98. *14 fig.* 1922.
3. KROUT, W. S. Combating apple scab. Spraying and dusting experiments in 1922. Massachusetts Agric. Exp. Sta. Bull. 214: 29–41. 1923.
4. PATTEN, A. J., and P. O'MEARA. The probable cause of injury reported from the use of calcium and magnesium arsenates. Michigan Agric. Exp. Sta. Quart. **2**: 83–84. 1919.
5. SAFRO, V. I. An investigation of lime-sulphur injury; its causes and prevention. Oregon Agric Exp. Sta. Res. Bull. 2. *32 p., pl. 4.* 1913.
6. SMITH, C. M. Excretions from leaves as a factor in arsenical injury to plants. Jour. Agric. Res. **26**: 191–194. 1923.
7. STEWART, J. P. Preparation and use of the concentrated lime-sulphur spray. Pennsylvania Agric. Exp. Sta. Bull. 115: *23 p., 3 fig.* 1912.
8. SWINGLE, D. B., H. E. MORRIS, and EDMUND BURKE. Injury to foliage by arsenical spray mixtures. Jour. Agric. Res. **24**: 501–538. *Pl. 1 (col.).* 1923.
9. WALLACE, ERRETT. Spray injury induced by lime-sulphur preparations. Cornell Agric. Exp. Sta. Bull. 288: 103–137. 1910.
10. YOUNG, H. C. The toxic property of sulphur. Ann. Missouri Bot. Gard. **9**: 403–435. 1922. Bibliography, p. 433–435.

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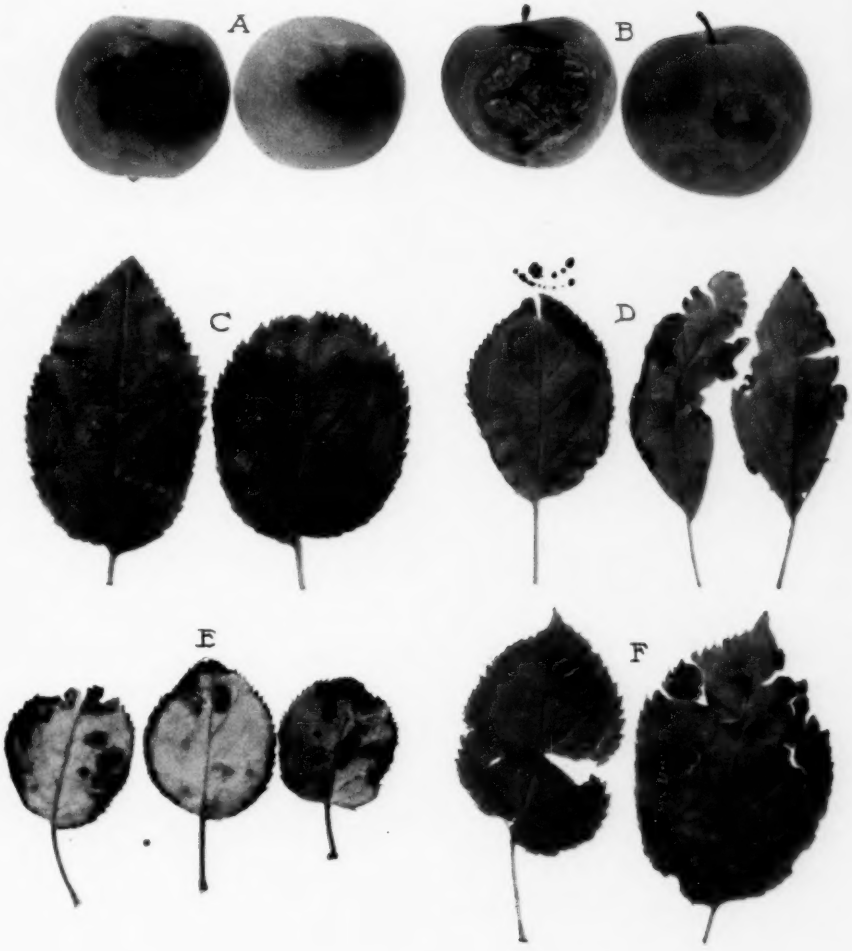

PLATE XIV. Various types of spray injury. A, B, and C.—Scald on fruit and leaves; D.—Edge burn; E.—Yellow leaf; F.—Injury following apple scab.

UNIV OF MICH.

A CYTOLOGICAL STUDY OF CERATOSTOMELLA FIMBRIATA (E. & H.) ELLIOTT[1]

JOHN A. ELLIOTT

WITH PLATES XV AND XVI

INTRODUCTION

The cytological study here recorded was undertaken as essential in confirming the decision that the so-called pycnidial stage of the sweet potato black-rot fungus is in reality a perfect or sexual stage (4). Due to the unusual nature and behavior of the asci, and to their small size, a study of the nuclear phenomena in the early stages of formation of the perithecia was thought necessary. The presence of sexual organs and nuclear fusion having been established, the study was continued in as much detail as the very minute size of the nuclei would permit. In addition to being of importance in establishing the true nature of the fungus studied, the phenomena observed are likely to be of interest in reference to the nuclear behavoir of Ascomycetes in general, especially as this fungus falls within a group of the Ascomycetes, the Sphaeriales, that has been little studied cytologically. *Gnomonia erythrostroma* was studied by Brooks (3), but its behavior is entirely different from that of *Ceratostomella fimbriata*.

The literature dealing with nuclear phenomena in the Ascomycetes is extensive, covering many different types exhibiting quite varied degrees of sexuality. This literature has been very well reviewed and interpreted by Atkinson (1) and Gwynne-Vaughn (5) from radically different points of view. It is my purpose in this paper to mention only such observations of others as may be directly compared with those made in this study.

METHODS OF PROCEDURE

The material used in this investigation was procured from a culture of the sweet potato black-rot fungus growing on the cut surface of sweet potatoes kept at room temperature in a moist chamber. The fungus developed normally, producing the typical black rot of the sweet potato. In about a month's time heavy fruiting of the supposed pycnidial form

[1] This manuscript was prepared by the late Doctor Elliott. After his death it was sent by Dr. Bradford Knapp, of Arkansas, to Dr. Thomas F. Manns, who, with Dr. W. J. Baerg, of Arkansas, finished certain details and submitted the paper for publication. While it is not as complete and conclusive as it probably would have been had Dr. Elliott lived, it is published with the idea that it may stimulate further work on the problem.—ED.

of the fungus was noted, and a considerable amount of the material was prepared for making permanent microscopic slides for class use. This work was done by Mr. Sam Poe, student laboratory assistant. The material was fixed in weak Flemming's solution and imbedded in the usual manner. Sections were cut from 3 μ to 7 μ in thickness, those of 4–5 μ proving the most satisfactory. Flemming's triple stain and Haidenhain's iron-alum haematoxylin without a counter stain gave good results.

In the early stages of the formation of the perithecium, the differentiation of the sexual elements, fertilization of the oogonium, and up to the development of the ascogenous hyphae, could be made out nicely from the triple stained slides. During these stages there was little complication in details. The nuclei were relatively large as compared with other elements and the cytoplasm was apparently dilute and did not interfere. Details of the nuclear behavior, from the formation of the ascogenous hyphae to the cutting out of the ascospores, could be followed most readily in sections stained with haematoxylin. This was due to the extreme minuteness of the nuclei in the ascogenous hyphae and the density of the cytoplasm in the developing asci. In spite of their minute size, the nuclei in all their stages stood out sharply and distinctly in the haematoxylin preparations. The nuclei in all stages were too small for the number or behavior of the chromosomes to be observed.

THE DEVELOPMENT OF THE ASCOCARP

Throughout its vegetative stage the sweet potato black-rot fungus is uninucleate. The hyphal cells and both forms of asexual spores clearly showed their uninuclear condition when stained with haematoxylin (Pl. XV, fig. 1). The morphology of these forms, which has been very well shown by Halsted (6) and Halsted and Fairchild (7), needs no amplification here. This uninucleate nature of the vegetative parts simplified the study of the nuclear phenomena of the fungus in the sexual organs and throughout the history of the perithecium, as will appear later.

Whether or not it is essential that the different sexual elements arise from distinct hyphal strands, in several instances two hyphal branches from different sources were observed to form the original hyphal knot from which the perithecium was to develop. Another condition which seems to favor the view of the different origin of the sexual elements is that the perithecia are always formed in groups, usually crowded together in narrow zones and without relation to the abundance of mycelium or of the asexual fruiting stages.

The branch which is to produce the oogonium becomes thickened at the base, the antheridial branch twists itself around this branch (Pl. XV, figs.

2–5) which differentiates into basal cell, oogonium and trichogyne. The nuclei at this stage become prominent and stain very readily, the triple stain being very effective in bringing out the different elements. The trichogyne is continuous with the oogonium and has no nucleus and no dividing wall. The trichogyne apparently fuses at any point it makes contact with the antheridium. A single nucleus from the antheridium passes through the trichogyne into the oogonium. This migration has been observed frequently and in all stages. The nuclei are very small and chances for differences in interpretation are obvious, as a survey of the literature on the subject will show. The simplicity of the sex organs of this fungus, however, makes it seem less likely that misinterpretations would be made here than in the case of some of the much more complicated nuclear phenomena occurring in some of the other oogonia that have been studied. Whatever may be the meaning of the nuclear fusion in the oogonium of *Ceratostomella fimbriata*, I can see no way of avoiding the conclusion that a fusion takes place. Practically all stages in the history of this fusion have been observed, and the oogonium, with a single large nucleus, has been found repeatedly in stages of the development of the perithecium far too late for the migration of the antheridial nucleus not to have occurred. The stages in the fertilization of the oogonium in *C. fimbriata*, except for the presence of the trichogyne, are very similar to those described and figured by Harper for *Sphaerotheca castagnei* (8), *Erysiphe polygoni* (9) and *Phyllactimia Corylea* (10), and by Blackman and Fraser for *Sphaerotheca castagnei* (2). The indications are that in many cases a considerable period elapses between the fusion of the two nuclei in the oogonium and the division of this fusion nucleus into the pairs of nuclei which migrate into the ascogenous hyphae (Pl. XV, figs. 6–11). When the fusion nucleus does divide, the division takes place within the nuclear-plasm (Pl. XV, figs. 12–13), and its first division is not likely to be confused at any stage with the fusion of the male and female nuclei or be interpreted as such, as has been suggested by Atkinson (1). Eight very small nuclei have been observed as the result of this division, but not enough observations have been made to determine whether this is a usual and fixed number.

During the resting stage of the fertilized oogonium the perithecium makes considerable growth. Immediately surrounding the oogonial cell, large thin-walled cells (Pl. XV, figs. 6–13, Pl. XVI, figs. 14–16) originating, apparently, from the unfertilized basal cell of the archicarp, partly fill the cavity of the rapidly growing perithecium. The walls of the perithecium are made up of vegetative cells originating from hyphal branches closely associated with the antheridial and oogonial branches.

Ascogenous hyphae arise from the carpogonium, the nuclei apparently migrating into it in pairs which divide simultaneously throughout the

growth of the hyphae. This is a very definite pairing and not, as Gwynne-Vaughn (5) has suggested for some cases, merely an apparent pairing due to rapid successive divisions of the nuclei before they have had time to separate (Pl. XVI, figs. 14–15). The ascogenous hyphae branch throughout the perithecial cavity between the large thin-walled sterile cells, which may occupy considerable of the space against the walls. In some instances the large sterile cells which usually line the cavity fail to develop and the perithecial cavity is entirely empty in its early stages of development until occupied by the ascogenous hyphae. The hyphae may grow around the inner walls of the perithecium or directly across it. Whenever the hyphae come in contact with the large sterile cells, or in the absence of these cells, with the thick-walled cells making up the true perithecial wall, they form a permanent contact with them and become more or less independent of the original base, *i.e.*, the oogonial cell (Pl. XVI, figs. 15–16). When the perithecium has reached the stage of development in which the mother ascus-cells are being differentiated from the hyphal branches, the hyphae appear to be arising from all parts of the inner wall of the perithecium and their primary point of origin is not discernible. It is apparent that the function of the large thin-walled cells is to act as nutrient cells to the ascogenous hyphae. The first strands of the ascogenous hyphae are very fine threads about $\frac{3}{4}$ μ in diameter, but the secondary branches, especially those with secondary attachments, are considerably larger (Pl. XVI, fig. 15). The nuclei are in all cases very minute and may be seen lying side by side even in the narrowest hyphal strands. Asci are first developed at the apex of the perithecium, from which the long beak is simultaneously being produced. The development of asci progresses backward toward the base and outer walls of the perithecium until the walls are reached and the ascogenous hyphae and the nutrient cells are exhausted. The perithecium finally collapses, although vegetative hyphae and the large asexual olive conidia have been observed growing out from the inner walls and partly filling the cavities.

THE DEVELOPMENT OF THE ASCI AND ASCOSPORES

As far as could be observed, the ascogenous hyphae were coenocytic; there did not appear to be even a cutting off, at least in the early stages, of the asci from the hyphae, although this probably occurs in the later stages of development. Nothing in the nature of an ascus hook is produced; the mother ascus-cell first appears as a terminal swelling of a branch of the ascogenous hyphae. It is at first bi-nucleate, which condition maintains until the cell has become about half the diameter of the mature ascus. During the growth of the mother ascus-cell the two nuclei also enlarge

greatly (Pl. XVI, figs. 16, 19, 22). The two nuclei fuse to form a single nucleus which is about half the diameter of the fusion nucleus of the fertilized oogonium. This fusion is followed almost immediately by a series of three divisions producing eight nuclei. As in the carpogonium, these divisions occur within the nuclear-plasm, which in many cases is distinctly separate from the cytoplasm of the ascus (Pl. XVI, figs. 16, 23, 25). The free nuclei are finally cut out of the cytoplasmic matrix in the manner first described by Harper (8) and later by many others (Pl. XVI, figs. 16, 23, 27). The presence of a centrosome could not be demonstrated, but the nucleus is drawn out to a point from which the enveloping wall of the spore develops, cutting out the spore. Either before or shortly after the completion of this process the ascus wall disintegrates and soon entirely disappears, leaving the spores (eight or less) more or less closely associated but without an enclosing wall. The spores may continue their development for some time after the dissolution of the ascus, finally attaining a size of 2.5–3.5 by 3–5 μ (Pl. XVI, figs. 28–29). No ascospores were observed in this study which measured more than 5 μ by the longest diameter. Apparently, the measurements given by Halsted and Fairchild (7) are of spores undergoing secondary enlargement, or they are the measurements of the developing asci, which they very closely approximate.

SUMMARY

Several phenomena of interest have been observed in the study of the cytology of *Ceratostomella fimbriata*. 1. There is a fusion of the male and female nuclei in the oogonium followed by a division producing eight nuclei, which migrate into the ascogenous hyphae in pairs. 2. The nuclei of the ascogenous hyphae undergo conjugate division throughout the growth of the hyphae until the ascus mother-cell is produced. 3. The ascus mother-cell is terminal and is not cut off from the hyphae by a wall, so far as has been observed. 4. The ascogenous hyphae fuse with the large uninucleate nutritive cells lining the wall of the perithecium and form new bases from which further development takes place. 5. The ascus disintegrates before the ascospores are entirely mature.

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LITERATURE CITED

1. ATKINSON, G. F. Phylogeny and relationships in the Ascomycetes. Ann. Mo. Bot. Garden **2**: 315–376. *10 figs.* 1915.

2. BLACKMAN, V. H., and H. C. I. FRASER. Fertilization in Sphaerotheca. Ann. Bot. **19**: 567–569, *fig. 17*. 1905.

3. BROOKS, F. T. The development of *Gnomonia erythrostoma* Pers., the cherry-leaf-scorch disease. Am. Bot. **24**: 585–605, *pl. 48–49*. 1910.

4. ELLIOTT, JOHN A. The ascigerous stage of the sweet potato black-rot fungus. (Abstract) Phytopath. **13**: 56. 1923.

5. GWYNNE-VAUGHN, DAME HELEN. Fungi, Ascomycetes, Ustilaginales, Uredinales. *232 p.*, illus. 1922.

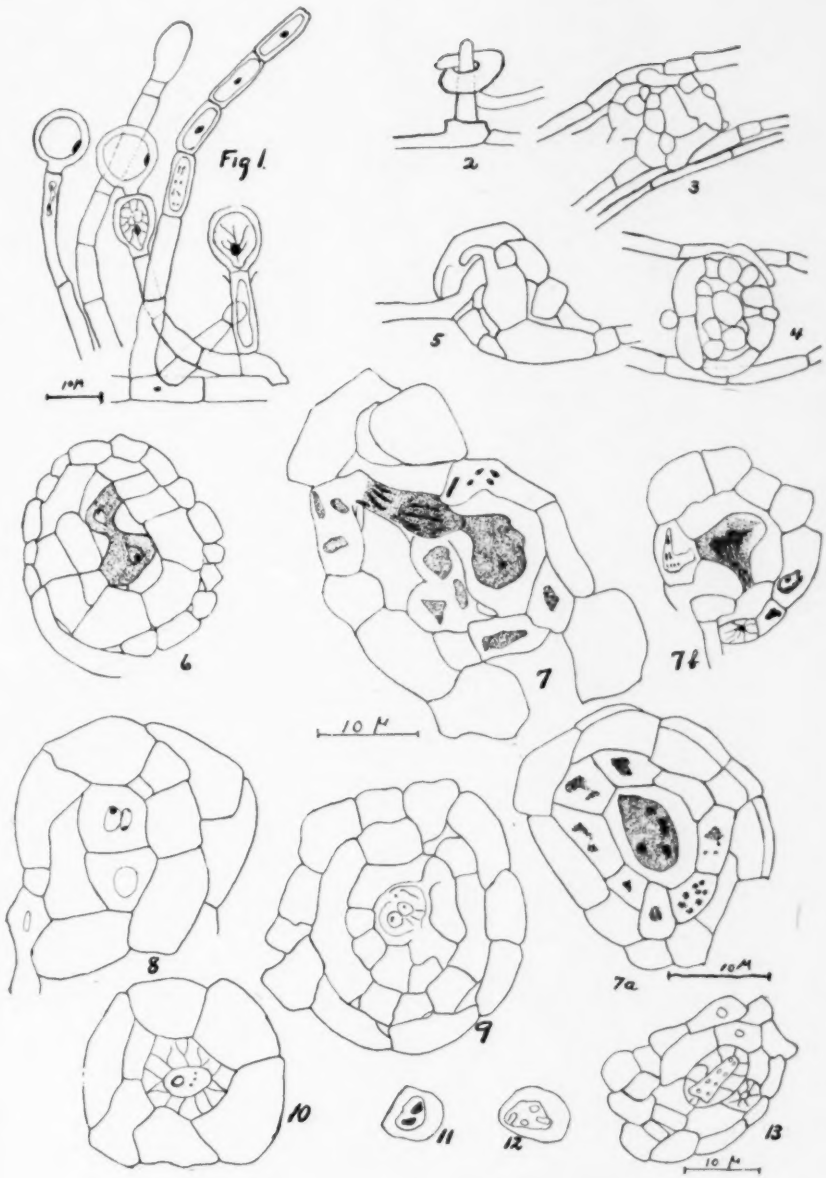
6. HALSTED, B. D. Some fungous diseases of the sweet potato. New Jersey Agric. Exp. Sta. Bull. 76. *32 p., fig. 3–7.* 1890.

7. ———————, and D. G. FAIRCHILD. Sweet potato black rot. Jour. Myc. **7**: 1–11, *pl. 1–3.* 1891.

8. HARPER, R. A. Die Entwickelung des Peritheciums bei *Sphaerotheca castagnei*. Ber. Deut. Bot. Gesell. **13**: 457–481, *pl. 39.* 1896.

9. ———————. Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten. Jahrb. Wiss. Bot. [Pringsheim] **29**: 654–685, *pl. 11–12.* 1896.

10. ———————. Sexual reproduction and the organization of the nucleus in certain mildew. *104* p., *7 pl.* 1905. (Carnegie Inst. Washington, Pub. 37.)



A CYTOLOGICAL STUDY OF CERATOSTOMELLA FIMBRIATA

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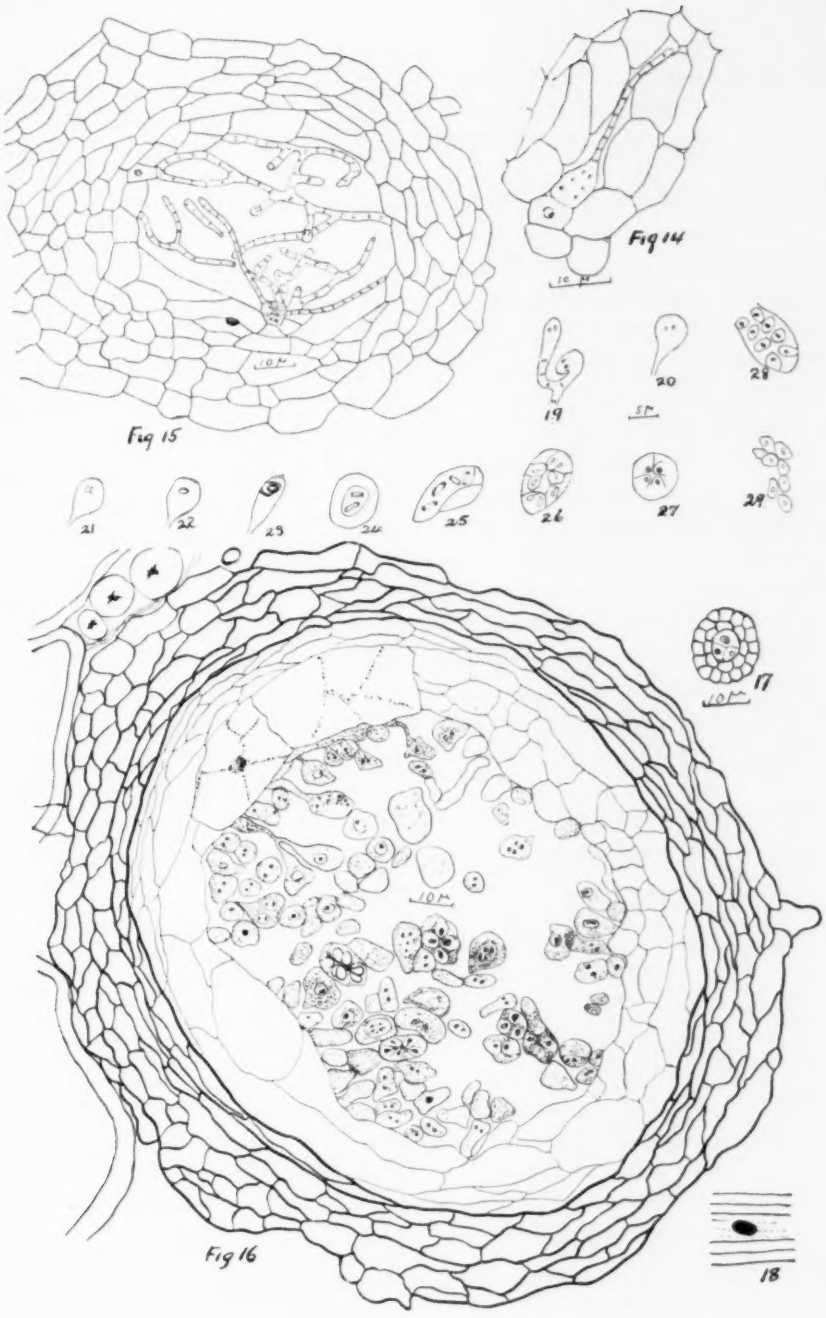

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VARIETAL RESISTANCE OF SWEET POTATOES TO NEMATODES, HETERODERA RADICICOLA (GREAF.) MÜLLER, IN CALIFORNIA

J. L. WEIMER and L. L. HARTER

It is a well-known fact that sweet potatoes are attacked by nematodes[1] and that some injury to the crop results.[2] That this pest is not considered of very great importance on this host probably explains why it has received scant attention as compared with that accorded other sweet-potato troubles. This fact is emphasized by Elliott,[2] who states that the reports by two growers in southern Arkansas of the total loss of their crop due to nematodes were unusual enough to be of interest to other pathologists.

In the spring of 1923 while investigating the diseases of sweet potatoes in California, considerable injury to the slips in the seedbed was observed on a ranch in Los Angeles County. The grower claimed to have lost his entire crop in certain parts of his field the preceding season. Nearby farmers claimed to have suffered considerable reduction to their crops from the same cause. Not only was it apparent that the nematodes were causing more or less direct loss to the sweet-potato crop in the fields, but the presence of large numbers of them in the seed potatoes and on the fibrous roots of the slips showed that sweet potatoes were a big factor in their distribution.

The examination of plants in the seedbeds and in the fields showed that nematodes were quite generally prevalent in sweet potatoes in other parts of the state.

That the nematodes can be distributed with infected seed was shown by the fact that when such seed was bedded in uninfested soil diseased plants resulted.

In one of the fields above referred to it was noted that nematode injury was much worse on some varieties than on others, which suggested the possibility of combating the disease by the use of resistant varieties. Seed potatoes of eight varieties of sweet potatoes (Red Brazil, Red Jersey, Southern Queen, Big Stem Jersey, Yellow Belmont, Nancy Hall, Porto Rico, and Yellow Jersey) were obtained for trial. The seed potatoes were treated in $HgCl_2$ (1:1000) for 10 minutes and then bedded at Garden Grove, Calif., March 5, 1924, in a soil known to be badly infested with nematodes. On

[1] Bessey, E. A. Root-knot and its control. U. S. Dept. Agr. Bur. Plant Ind. Bul. 217: 1–89. 1911.

[2] Elliott, J. A. Nematode injury to sweet potatoes. Phytopath. 8: 169. 1918.

May 1, when slips were pulled for planting, a few galls were found on the fibrous roots of some of the Red Brazil plants only. That nematodes were present in the soil and that conditions were favorable for infection were denoted by the presence of large galls on the roots of tomato plants set in different places in the bed at bedding time. However, on May 27, when a second pulling of plants was made, numerous galls were found on the roots of both the Nancy Hall and Red Brazil plants. A few very small galls were found on several plants of the Southern Queen and Yellow Belmont varieties. None were found on any of the other varieties. 200, 100, and 50 plants of each variety were planted on infested soil at Garden Grove, Baldwin Park, and Santa Ana, Calif., respectively. These were planted in commercial sweet-potato fields alongside of the commercial crop and given the same care.

Very little difference in the appearance of the plants of the different varieties could be detected, except possibly in the Nancy Hall variety at Garden Grove, in which case the plants were small and lacking in vigor. The plots were dug October 13, 14, and 15, at which time observations were made on the number of galls on the roots of each plant and the yield of each variety. These data are given in tables 1 and 2.

TABLE 1.—*The degree of infection of the different varieties of sweet potatoes grown on the different plots*

Variety	Baldwin Park	Santa Ana	Garden Grove
Porto Rico	Found only about a dozen galls	No galls seen	No galls seen
Big Stem Jersey	No galls seen	A few galls seen	About half a dozen galls seen
Little Stem Jersey	" " "	" " " "	Few nematodes in base of stem; none seen on fibrous roots
Red Jersey	" " "	No galls seen	No galls seen
Southern Queen	" " "	" " "	" " "
Yellow Belmont	" " "	" " "	" " "
Nancy Hall	Numerous galls on fibrous roots. Larger roots rough and irregular. No roughening of potatoes	Numerous galls on fibrous and larger rootlets as well as in the base of the stem	Galls very abundant. Small and large roots deformed and decayed. Root systems very meager. Only a few small potatoes produced
Red Brazil	About same as Nancy Hall	Numerous small galls and swollen rootlets	Numerous galls on fibrous roots. Main stem attacked and considerably roughened

TABLE 2.—*The number of hills, total weight, and weight of marketable potatoes produced; also the average weight per hill of potatoes produced by each variety on the different plots*

	Plot	Varieties							
		Porto Rico	Southern Queen	Red Jersey	Yellow Belmont	Red Brazil	Nancy Hall	Big Stem Jersey	Little Stem Jersey
Number of hills	Baldwin Park	48	47	41	44	47	27	48	39
	Santa Ana	46	75	58	44	44	44	47	39
	Garden Grove	173	189	194	199	160	152	192	190
Total number lbs.	Baldwin Park	75.5	49.5	48	45	44	43.5	53.5	37
	Santa Ana	32	52.5	31	25.5	31	21.5	32	23.5
	Garden Grove	243	236	297.5	182.5	162	77.5	169	183.5
Total number lbs. marketable potatoes	Baldwin Park	66.5	44.5	36	39	32.5	37	32.5	24
	Santa Ana	25.5	37	19	17.5	24.5	12	20	12.5
	Garden Grove	214	199	217.5	141	140.5	59	109.5	111.5
Average No. lbs. marketable potatoes per hill	Baldwin Park	1.39	0.95	0.88	0.89	0.69	1.37	0.68	0.62
	Santa Ana	0.55	0.49	0.33	0.40	0.56	0.27	0.43	0.32
	Garden Grove	1.24	1.05	1.12	0.71	0.88	0.39	0.57	0.59

An examination of table 1 shows that at digging time no nematode galls were seen on any of the plants of the Red Jersey, Southern Queen, or Yellow Belmont varieties. A few galls were found on a few plants of the Little Stem Jersey, Big Stem Jersey, and Porto Rico in some of the plots. On the other hand, the Nancy Hall and Red Brazil varieties were badly infected in all plots. In the case of the two latter varieties the galls were sufficiently numerous to cause a considerable decrease in yield. Owing to the various sources of error in plot experiments of this kind and size, the decrease in yield due to nematodes in these varieties could not be accurately determined from the data at hand. However, the figures in table 2 indicate a considerable reduction in yield of the Nancy Hall variety in the plots at Santa Ana and Garden Grove, especially when compared with the Porto Rico. In the plot at Baldwin Park the Nancy Halls yielded about as well as the Porto Rico; in fact, in this plot, although the plants were fairly heavily infected, the nematodes seemed to have little effect on the yield. A

more vigorous vine growth was made in this plot, indicating that conditions for plant growth were such that the infected varieties were able to produce a good crop of potatoes in spite of the nematodes. In the plots at Santa Ana and Garden Grove, on the other hand, poorer conditions for vine growth prevailed, resulting in a correspondingly greater loss due to the ravages of the nematode. This may explain why the Nancy Hall variety grown on infertile, light, sandy soil, heavily infested with nematodes, is frequently a total loss, while when grown on fertile soil, even though nematode-infested, it may produce a fair or even a good crop. However, it is questionable if it is ever advisable to attempt to grow a crop so susceptible to nematodes as either the Nancy Hall or Red Brazil varieties of sweet potatoes on nematode-infested soil. Not only is there the risk of not obtaining a crop, but that of increasing the nematodes in the soil, so that other susceptible crops cannot be grown profitably. The growing of susceptible varieties may also be the means of carrying the nematodes on the plants to uninfested fields. The Red Jersey, Little Stem and Big Stem Jerseys, Porto Rico, Southern Queen, or Yellow Belmont varieties, while not immune, are highly resistant and might be substituted for the more susceptible varieties on nematode-infested land. The more resistant varieties of sweet potatoes can be used to supplant other more susceptible crops on sandy land infested with nematodes.

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POTATO LEAF ROLL AS AFFECTING THE CARBOHYDRATE, WATER, AND NITROGEN CONTENT OF THE HOST

ELMER GRANT CAMPBELL[1]

WITH THREE FIGURES IN THE TEXT

It has been well established by qualitative tests that potato leaf roll is accompanied by an accumulation of starch in the leaves of the host. The earlier literature on this line of work has been reviewed by Murphy (5). Without restating his report it may be sufficient to note that all the references cited by him having a bearing upon the work reported herein have been examined. Approximately all efforts thus far have been not only qualitative in outlook but have pointed solely toward the location of starch.

Esmarch (2), Neger (6), Hiltner (4), and Murphy (5), by the use of the iodine test, have shown that leaf-roll disease is characterized by an accumulation of starch in the leaves. Neger has also shown that diseased leaves are low in water content. Boas (1) found by quantitative methods that the stems of diseased plants were high in amino acids. He found also that in leaf-roll plants there was a higher catalase content—and a higher hydrogen-ion concentration.

The purpose of this short paper is to set forth the results of a brief quantitative study of the carbohydrate, water, and nitrogen contents of leaf-roll potato plants as compared with healthy potato plants.

TABLE 1.—*Dry Weight and C/N Ratio*

Variety	Condition	Percentage dry wt.	Percentage total carbohydrate*	Percentage total nitrogen*	C/N ratio
Rural New Yorker	Healthy	6.79	10.80	5.99	1.80
(Age, 5½ weeks)	Leaf roll	9.49	25.01	4.67	5.35
Irish Cobbler	Healthy	8.94	17.56	3.15	5.57
(Age, 11 weeks)	Leaf roll	10.35	26.75	4.28	6.25
Early Ohio	Healthy	7.17	4.47	5.57	.80
(Age, 11 weeks)	Leaf roll	9.73	16.40	5.50	2.98

* Figured on basis of dry weight.

[1] The author wishes here to express his indebtedness to Dr. Max W. Gardner and Mr. James B. Kendrick for the plants used in these enalyses, to Mr. P. H. Brewer for assistance in laboratory technique, and to Dr. Gardner for helpful suggestions in the preparation of the manuscript.

MATERIALS AND METHODS

Three varieties of potatoes were used in this experiment: Rural New Yorker, Irish Cobbler, and Early Ohio. Healthy and leaf-roll plants were grown under identical greenhouse conditions. Before the time of tuber formation, the plants were clipped at the surface of the soil (healthy and diseased at the same time), weighed, and immediately crushed in 95 per cent alcohol, to which calcium carbonate had been added in amount of one gram per liter. The pulp was placed inside an electric oven and brought to a constant dry weight. It was then ground to a fine powder and used for quantitative determinations as follows: twelve grams for the reducing sugars, two grams for the polysaccharides, and two grams for the total nitrogen. For the determination of the reducing sugars and the non-reducing sugars the official Fehling solution method was used. For the polysaccharides the official hydrochloric acid method of hydrolysis was used, after which the determinations were made by the same method as for the sugars. The total nitrogen was determined by the usual Kjeldahl-Gunning method. All percentages were figured on the basis of dry weight.

TABLE 2.—*Carbohydrates in Percentage of Dry Weight*

Variety	Condition	Reducing sugar	Non-reducing sugar	Polysaccharides
Rural New Yorker (Age, 5½ weeks)	Healthy	0.60	0.40	9.81
	Leaf roll	1.30	1.66	22.05
Irish Cobbler (Age, 11 weeks)	Healthy	0.68	0.31	16.57
	Leaf roll	1.38	1.70	23.67
Early Ohio (Age, 11 weeks)	Healthy	0.10	0.27	4.10
	Leaf roll	1.15	1.80	13.45

RESULTS

Invariably the leaf-roll plants were higher in percentage of dry weight. This fact is shown graphically in figure 1, and numerically in the dry weight column of table 1. The ratio of carbohydrate to nitrogen runs consistently higher in the diseased plants, as is shown in the right-hand column of table 1 and by fig. 3. All forms of carbohydrate, reducing sugars, non-reducing sugars, and polysaccharides, are higher in the diseased plants. The data are given in table 2. The total carbohydrate in healthy as compared to diseased plants is shown graphically in figure 2.

SUMMARY

Let it be emphasized here:

1. That the plants involved in this study were grown under identical greenhouse conditions.

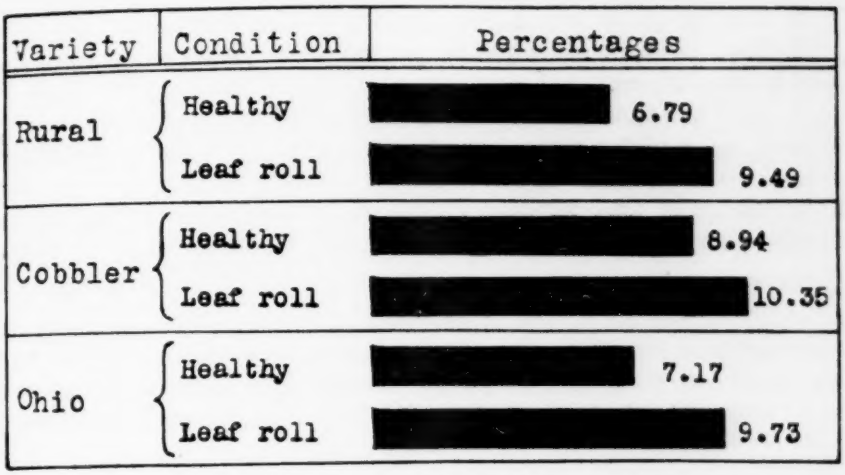

FIG. 1. Dry weight ratio.

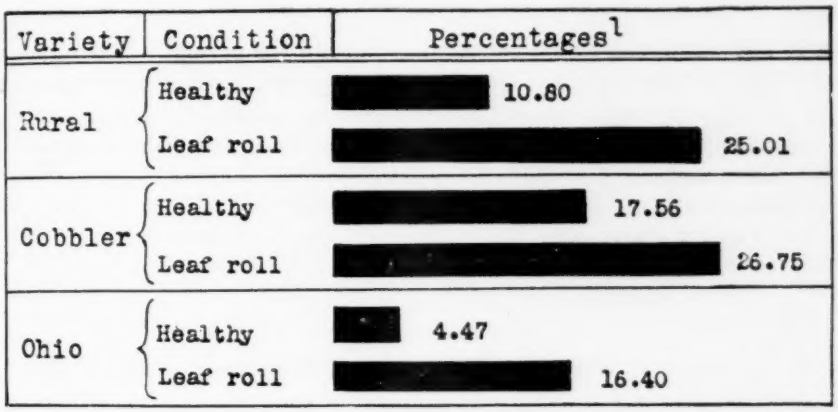

FIG. 2. Carbohydrate content. [1] Figured on basis of dry weight.

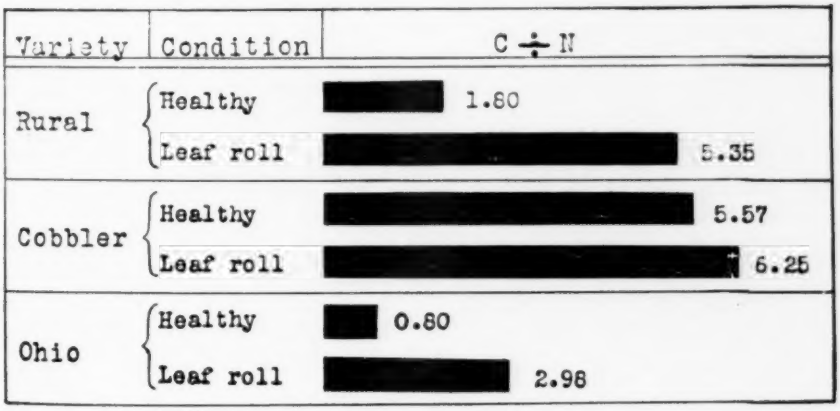

FIG. 3. Carbohydrate-nitrogen ratios.

2. That the plants were harvested prior to tuber formation.

3. That the entire above-ground portion of the plants was crushed and used in the analyses.

4. That in comparison with the healthy, the leaf-roll plants invariably showed: (a) a higher percentage of dry weight; (b) a higher percentage of carbohydrates, sugars as well as starch; (c) a higher C/N ratio; (d) and a percentage of total nitrogen approximately equivalent to that of the healthy plants on the basis of dry weight, and higher on the fresh weight basis.

REMARKS

From these facts it does not seem probable that a retarded translocation rate is the only factor causing a simultaneously high carbohydrate and low water content in the leaf-roll plants. A high sugar content under conditions of abundant external water should normally mean a correspondingly high water content for the plant, but in this instance such is not the case. It may be that the disease virus in leaf-roll plants has retarded the movement of the transpiration stream, or that possibly it has somehow stimulated photosynthetic action temporarily beyond the normal. The operation of either of these assumed possibilities or a combination of them might change the dry weight-carbohydrate ratio to an abnormal figure.

Further studies of these physiological processes are being planned in this laboratory.

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LITERATURE CITED

1. BOAS, FRIEDRICH. Beitrage zur Kenntnis des Kartoffelabbaues. Ztschr. Pflanzenkrank. 29: 171–176. 1919.
2. ESMARCH, F. Zur Kenntnis des Stoffwechsels in blattrollkranken Kartoffeln. Ztschr. Pflanzenkrank. 29: 1–20. 1919.
3. GARDNER, MAX W., and JAMES B. KENDRICK. Potato leaf roll in Indiana. Purdue University Agr. Exp. Sta. Bul. 284: 1–23. 1924.
4. HILTNER, L. Versuche uber die Ursachen der Blattrollkrankheit der Kartoffel. II–Weitere Beobachtungen uber die "Stärkeschoppung" in blattrollkranken Kartoffelstauden. Praktische Blätter fur Pflanzenbau und Pflanzenschutz. (München.) 17: 15–19. 1919.
5. MURPHY, PAUL A. On the cause of rolling in potato foliage; and on some further insect carriers of the leaf-roll disease. Sci. Proc. Roy. Dublin Soc. 17: 163–184. 1923.
6. NEGER, F. W. Die Blattrollkrankheit der Kartoffel. Ztschr. Pflanzenkrank. 29: 27–48. 1919.

PHYTOPATHOLOGICAL NOTES

Taphrina communis and Taphrina mirabilis. My attention has been called to the fact that Giesenhagen (Die Entwickelungsreihen der parasitischen Exoasceen. Flora **81**: 267–361. 1895) used the combinations *Taphrina communis* and *T. mirabilis* for the species described by Sadebeck and Atkinson, respectively, as *Exoascus communis* and *E. mirabilis.* In my paper in the February issue of Phytopathology (Cultural and morphological studies of some species of *Taphrina*), therefore, the specific names in question should appear as *Taphrina communis* (Sadeb.) Giesenhagen and *T. mirabilis* (Atkinson) Giesenhagen.—Ella M. Martin, Illinois Wesleyan University, Bloomington, Illinois.

BOOK REVIEWS

Laboratory Outlines in Plant Pathology. By H. H. Whetzel, L. R. Hesler, C. J. Gregory, and W. H. Rankin. Second Edition. 231 pages. W. B. Saunders Co. 1925.

The contents of this work bear out the announcement that the second edition of this valuable laboratory guide has been "completely revised and rewritten by the senior author." When Professor Whetzel proposed to turn over to the younger men the chief executive responsibilities of the Cornell Department of Plant Pathology in 1922, he stated that he wished to give part of the time thus freed to book-making. While all American teachers of applied botany are awaiting with especial eagerness the appearance of his promised new "text book," we welcome meanwhile this careful revision of the laboratory outlines. Probably in no other laboratory have so many of these diseases received first hand investigation. Hence the outlines in every chapter may be taken as authoritative guides checked against long experience with classroom use.

The new publishers have given it a clean, attractive make-up, and it bears throughout evidence of careful attention to typographical form and proof-reading. The general plan, as one would of course expect, follows that of the first edition, grouping the examples under the three headings, necrotic, hypoplastic, and hyperplastic diseases. The writer pointed out in his review of the first edition (PHYTOPATH. **8**: 6. 1918) that this sequence of laboratory exercises represents the unique experimental contribution of this book in the field of plant pathological teaching. Since most of us school teachers are peculiarly apt to become slaves to usage and easy habit followers, it is well to have a live-wire progressive like Whetzel jar us into a realization of the fact that success in teaching depends little on form or sequence of topic and much on vitalizing energy and contagious enthusiasm. Moreover, as stated in the preface, there are here included practically three times as many exercises as will ordinarily be covered in the usual undergraduate course in plant pathology, hence each teacher must select those exercises best suited to personal use. The mode of treatment is so admirably standardized and the exercises have such uniformity in mode of treatment that it seems to the reviewer possible for each teacher to take them up in almost any order he may wish. Some might criticize these outlines as lacking in organized effort at the comparative or progressive development of the topic which is typical of our better elementary laboratory courses in biology. The experience of the writer would indicate that plant pathology does not impose upon the teacher the same logical "simple to complex" sequence. Consequently, is it one of the many commendable features of these outlines that they present the wholesomely stimulating challenge to the teacher to formulate and develop his own central theme? As the authors wisely state in the Preface, "Although the acquisition of a body of facts is an important and necessary part of such a course, a more vital feature is the training in logical methods of acquiring them." It is evident from the general directions (pp. 11–28) that with the authors these would include training in critical comparison of "symptoms" and "signs" and in the use of literature. The preparation of a bibliography is one of the features where wholesome training in exactness is indicated by the detailed outline (pp. 13–17). If a suggestion were to be added by the reviewer to these already full directions it would deal with two points in which students commonly

need guidance and in which the editing of the reference lists of the body of the text is not wholly consistent (*e.g.*, compare the "References" cited, pp. 37, 43, 53, etc.). These concern the questions of whether or not one should or should not capitalize the leading words in cited titles and whether, in such titles, the binomial plant names should or should not be italicized. Unquestionably this training in exactness is favored also by the carefully prepared "glossary." It is to this, probably, that most mature phytopathologists will turn first to seek helpful additions or more refined distinctions for our vocabulary. As supplementing certain useful terms (inoculum, epiphytotic) which the first edition did much to fix in American usage, we find here some interesting additions. Especially one notes "suscept" as differentiated from our familiar "host" and "physiogenic" as applied to "a disease primarily caused by some non-living factor." Both of these certainly point to weaknesses in our technical terminology. Probably their acceptance will be further advanced by their usage as exemplified in the anticipated text-book. Meanwhile, the writer wishes to congratulate and thank the authors for their virile leadership in the educational aspects of our profession.

L. R. JONES

Diseases of Crop-plants in the Lesser Antilles. By William Nowell. Published on behalf of the Imperial Department of Agriculture by the West India Committee, London. 383 pages, 150 figures. 1923.

Mr. Nowell has given to planters in tropical regions a most comprehensive, lucid, and practical guide for the recognition and combatting of crop diseases, and he has given to students of plant pathology everywhere a most stimulating and helpful discussion of fundamental scientific principles.

The first part of the work discusses the nature and causation of disease, parasitism, and host resistance and susceptibility; then follows a condensed general outline of causal agents, a few pages being devoted to each of the principal parasitic groups of fungi, with chapters on pathogenic bacteria, infective viruses, phanerogamic parasites, nematodes, insects in their relation to plant diseases, non-parasitic diseases, and entomogenous fungi. General means of prevention and control are treated under such headings as spraying, dusting, disinfectants, wound treatment, elimination methods, disease resistance, and plant disease legislation.

The greater portion of the book is devoted to specific diseases of tropical crops. The treatment of each major disease gives a clear understanding of the symptoms and nature of the disease, the conditions influencing its development, and the most approved control measures. References to special literature are well selected, but include very few more recent than 1920. This is explained by the fact that the work was written during the author's tenure of the position of Mycologist on the staff of the Imperial Department of Agriculture for the West Indies (1913–1920), and there has been some delay in publication. As Prof. J. Bretland Farmer has well said in the foreword: "Mr. Nowell has embraced the opportunity which lay to his hand and has produced a work which in my judgment constitutes a real landmark of progress in the science of the plant, considered in relation to health and disease."

H. R. FULTON

The June number of Phytopathology was issued July 15, 1925.